Documentation related to Sessions 7 and 8 of the meeting Click on the links below to see the pre reads and presentations

Malaria Policy Advisory Committee (MPAC) Meeting, 2–4 October 2019

Documentation related to Sessions 7 and 8

Thursday, 3 October 2019			
	Session 7	**Open**	**For decision**
13:45 – 14:30	Update on the technical consultation on genomic surveillance Background | Presentation	Dr Laura Anderson	
14:30 – 15:15	Update on the technical consultation on *Anopheles stephensi* Background | Presentation	Dr Jan Kolaczinski	**For information**
15:15 – 15:45	Revision of WHO classification of glucose-6-phosphate dehydrogenase (G6PD) variants and International classification of diseases (ICD)-11 Background | Presentation	Dr Andrea Bosman	**For guidance**
15:45- 16:15	*Coffee break*		
	Session 8	**Open**	
16:15 – 17:15	Update on the E-2020 and STOP-malaria Background | Presentation	Dr Kim Lindblade	**For information**

** Provisional programme and may be subject to change*

Malaria Policy Advisory Committee Meeting
2–4 October 2019, Geneva, Switzerland
Background document for Session 7

Technical consultation on the role of parasite and anopheline genetics in malaria surveillance

5–7 June 2019, Geneva, Switzerland
Revised following the MPAC meeting which was held on the 2-4 October 2019

This document was prepared as a pre-read for the meeting of the Malaria Policy Advisory Committee and is not an official document of the World Health Organization.
WHO/CDS/GMP/MPAC/2019.17

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List of acronyms

AFP	acute flaccid paralysis
COI	complexity of infection
DRC	Democratic Republic of the Congo
EEMS	estimated effective migration surfaces
GMP	Global Malaria Programme
GPEI	Global Polio Eradication Initiative
GPLN	Global Polio Laboratory Network
IBD	identity/identical by descent
IBS	identity/identical by state
IRS	indoor residual spraying
ITN	insecticide-treated net
kdr	knock-down resistance
LLIN	long-lasting insecticidal net
MDR-TB	multidrug-resistant tuberculosis
MFO	mixed-function oxidase
MPAC	Malaria Policy Advisory Committee
NMCP	national malaria control programme
PAHO	Pan American Health Organization
PBO	piperonyl butoxide
PCR	polymerase chain reaction
PLA	plasmepsin amplification
QA	quality assurance
QC	quality control
RDT	rapid diagnostic test
SNP	single nucleotide polymorphism
TB	tuberculosis
TES	therapeutic efficacy study
WGS	whole genome sequencing
WHO	World Health Organization

1. Introduction

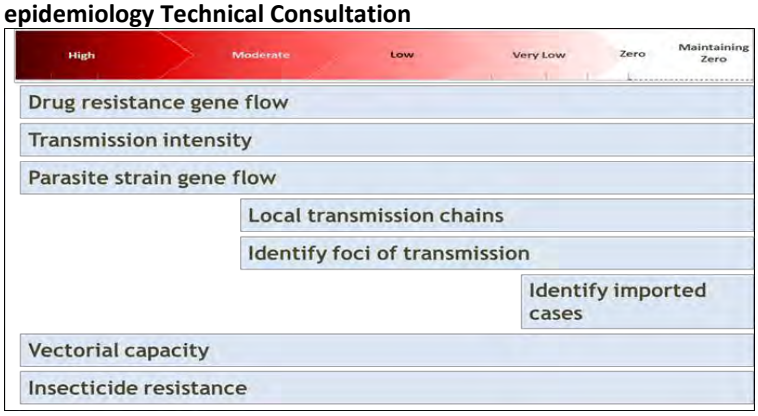
Advances in genetic epidemiology are creating new opportunities for the surveillance, prevention and control of infectious diseases. Emerging evidence shows that mosquito genotyping can improve the understanding of mechanisms of speciation and the processes that influence the mosquitoes' ability to transmit malaria parasites to humans. Such knowledge can foster a better understanding of vectorial capacity and consequently how to better target interventions. Research on parasite genotyping also indicates potential applications in the understanding of parasite gene flow, including drug-resistance genes, *Pfhrp2/3* deletions, quantification of malaria importation risk, as well as characterization of changing transmission intensity. Most of the work in malaria genetic epidemiology, however, has remained within the realm of research and has not been guided by clearly defined policy-relevant questions. There have been few examples of how such work could improve the operational decisions made by national malaria programmes.

For these reasons, the WHO Global Malaria Programme (GMP) convened a three-day Technical Consultation from 5 to 7 June 2019. The aim of the Technical Consultation was to review the existing evidence from genetic epidemiological research studies and use cases[1], and assess the role of such research in the development of future policies and the potential for malaria programmes to make practical use of genomics. The Technical Consultation also aimed at establishing a list of global research priorities for the future strategic use of genetic epidemiology, in the hopes of accelerating progress towards achieving the goals of the Global Technical Strategy for malaria *(1)*. The Technical Consultation was approved by the Malaria Policy Advisory Committee (MPAC) during its October 2018 meeting and was jointly convened by the GMP Units responsible for Surveillance, Monitoring and Evaluation (SUR); Drug Efficacy and Resistance (DER); Entomology & Vector Control (EVC); and Elimination (ELI). The meeting was chaired by Professor Dyann Wirth.

2. Objectives and expected outcomes

The main objectives of the consultation were to understand the role of genetic epidemiology (specifically parasite and anopheline genetic signals and gene flow) in malaria surveillance and control, and to define priority research questions that are relevant to policy and operational activities of national programmes (see Fig. 1).

Fig. 1. Topics across the transmission continuum recommended by MPAC for discussion during the genetics epidemiology Technical Consultation



Specifically, the consultation served to:

- review existing evidence across the use cases of genetic epidemiology in malaria surveillance;

[1] Research studies where genetic data has been used to understand parasite or mosquito gene flows and also have the potential to be used in malaria surveillance

- identify key research questions relevant to policy and operational activities of national programmes for each use case;
- Discuss appropriate study protocols and issues related to ethics, data sharing and coordination mechanisms.

Expected outcomes were:

- a meeting report summarizing the content of the presentations, discussions and outcomes of the meeting;
- a list of key research questions relevant to policy and operational activities of national programmes for each use case;
- a work plan to implement the key action points of the meeting.

This report summarizes:

- presentations given by meeting participants
- major discussion points
- the list of key research questions relevant to policy and operational activities of national programmes for each use case related to the topics of transmission and resistance
- next steps.

3. Summary of presentations and associated discussions

All presentations can be found at the following link:
https://www.dropbox.com/sh/zsuu5p3ls7m2l6w/AAAqO-Jfa0f73wXTBRpp-Puga?dl=0

3.1. The current and potential future role of genetic epidemiology in malaria surveillance

Presenter: Abdisalan Noor, WHO-GMP

Within WHO-GMP, work is ongoing in the use of molecular epidemiology for monitoring drug and insecticide resistance and the *pfhrp2/3* parasite gene deletions that evade detection by rapid diagnostic tests (RDTs). Data from monitoring sites and research studies around the world are displayed on the Malaria Threats Map[2], which provides a global spatial and temporal overview of vector insecticide resistance, parasite drug resistance and parasite *pfhrp2/3* gene deletions.

With the growing acceptance that genomics could play an integral role in policy and programmatic decisions, there have been increased investments, demonstration studies and refinement of sampling and analytical methods that could prove optimal in expanding the use of genomics as a tool in malaria control. There are, however, still significant unresolved issues related to priority research questions, programmatic applications, ethics of use of genetic material, data sharing and data use. A review of the range and complexity of genomic methods is also required to assess whether such methods are comparable and representative in different geographical contexts, and to determine the feasibility of implementation in countries with limited resources and limited capacity for data generation and analysis. Additionally, the fragmentation of genomics research has resulted in very few joint genetic and epidemiological analyses, which could provide practical applications for operational use and translation into policy. Without clear guidance on priority policy-relevant research questions, most of the studies may not have immediate policy relevance. It is our hope that this Technical Consultation

[2] The Malaria Threats Map is an online mapping platform that collates information on the biological challenges to malaria control and elimination, including insecticide and drug resistance, and gene deletions. The Malaria Threats Map App is available at: https://apps.who.int/malaria/maps/threats/.

will discuss these issues and identify evidence that could immediately contribute to policy recommendations, along with evidence that may be relevant but is only likely to be available within medium (five years) and long (10 years) timeframes.

3.2. Session 1: Experiences from other diseases: polio, Ebola and tuberculosis

Opening remarks by facilitator, Dyann Wirth

Genomic data have been applied to understanding the epidemiology of various infectious diseases, ranging from support for outbreak investigations to providing the foundation for elimination programmes. Understanding the practical application of genomics by other disease control programmes can offer insight into the potential uses and challenges in implementation at the national and subnational levels. The lessons learned from polio, Ebola and tuberculosis (TB) were presented in order to stimulate further discussion on potential use cases for malaria genomic epidemiology.

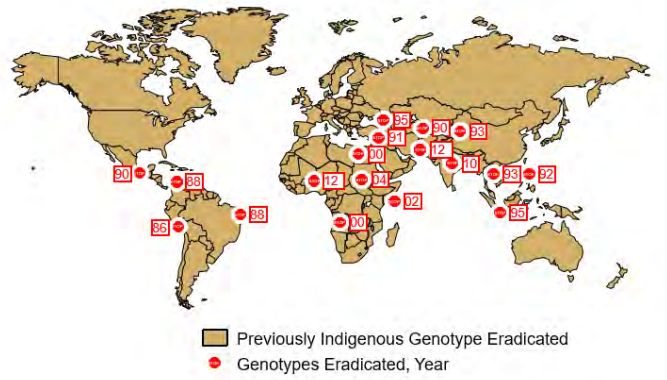
3.2a. Genetic epidemiology and disease surveillance for elimination: polio

Presenter: Ousmane Diop

Applications of genetic epidemiological methods have been a critical component in the success of the Global Polio Eradication Initiative (GPEI). The combination of an effective vaccine and strong collaboration between field disease surveillance and laboratory virologic surveillance teams has been crucial in achieving progress in the eradication of polio. Acute flaccid paralysis (AFP) surveillance, environmental surveillance, and special targeted studies within the control programme framework have allowed for strategic use of genetic epidemiology to determine the source of infection and inform whether transmission has occurred. In clinical surveillance, a suspected case has a sample sent for culture. Polymerase chain reaction (PCR) is used for intratypic differentiation, and any non-Sabin-like or indeterminate virus is sequenced. Genotypic indicators can then offer insight into transmission, including characterization of new virus introductions, epidemiological linkages between cases and surveillance quality. Environmental surveillance, including in areas where wild-type virus transmission has been interrupted but vaccine-derived virus transmission persists, can shed light on regional migration and flow patterns of the virus.

Inclusion of genetic epidemiology in the eradication initiative has provided a mechanism for accurately measuring key programme indicators: reduction in number of cases, geographic extent and genetic diversity. Identifying reductions in genetic diversity speaks to the overall progress towards eradication but requires knowledge of both the natural existing reservoir of viruses (so that new virus types can be identified) and the origin of introduced viruses. In 1988, there were over 30 identified genotypes and three serotypes circulating globally. Two serotypes were eradicated in 1999 and 2012: wild-type poliovirus (wt-PV) type 2 and type 3, respectively (Fig. 2). Only two genotypes remain in circulation: the SOAS genotype circulating in Afghanistan and Pakistan (most recent case in May 2019) and the WEAF-B1 genotype, which was last detected in Nigeria in 2016.

Fig. 2. Eradication of WPV3 genotypes, 1986–2012



Similarly, reduction and disappearance of genetic clusters represent progress regionally and locally for control programmes. Clusters include isolates sharing ≥5% of VP1 identity. For example, in 2018, six distinct genetic clusters were detected from AFP cases and environmental samples from the SOAS genotype. Expansion and reduction of genetic clusters are linked to transmission reservoirs, indicator communities and cross-border transmission, and vary with seasonality and peak transmission seasons. As surveillance quality indicators, genomic data have been used to identify orphan viruses (>1.5% different from the closest matching VP1 capsid sequence), which are indicative of possible missing cases in the transmission chain. Such data can also inform local population targets for improved vaccination campaigns. Furthermore, genomic data have been used as a mechanism for quality assurance and quality control (QA/QC) by identifying contaminants and providing evidence on mismanagement of samples in order to facilitate improvements in surveillance protocols and data management. The successful integration of genomic data into the polio eradication initiative has been in part due to the comprehensive understanding of the poliovirus molecular clock, including the rate of natural evolution, which allows for accurate classification of nucleotide divergence in isolates to discern genetic lineages and chains of transmission.

Despite the successful use of genomic data at the local, regional and international levels of collaboration, the programme is not without operational challenges. Key operational challenges and areas with opportunity for improvement include capacity, utility, meaningful collection and use of data, quality of information, coordination and data sharing. The quality and use of sequence information are dependent on the quality of all aspects of surveillance. Within the Global Polio Laboratory Network (GPLN), there is still a need for increased capacity in sequencing capabilities, including standardization of methodologies, training, and QA/QC. Coordination and data sharing occur between WHO (three levels), national ministries of health, and other organizational partners. However, acceptance of genetic epidemiology data and use as part of routine surveillance and decision-making requires that data sharing be comprehensive and decision-making consensual. The GPEI is structured around the WHO regions and a laboratory network (GPLN) that has the necessary capabilities for conducting the molecular work that supports the programme. Information exchange is critical and considered a significant success of the programme. This example highlights the need for timeliness in communication and in the management and movement of samples to appropriate laboratories within the network in order to allow for genomic data generation and analysis. Timeliness is highly dependent on local capacity. Generally, sequencing can be completed within one month of sample arrival, although in countries such as Pakistan where there is local capacity for molecular work, sequencing can be completed within a few weeks. Despite the time needed for sequencing, information exchange occurs in “real-time”. Current gaps in data sharing and the availability of whole genome data to support interpretation of locally generated data remain a concern, as such gaps can delay the use of information in decision-making. Ideally, a full database of all virus isolations that is shared and managed collectively would ensure the availability of data for accurate interpretation in a timely manner. Maintenance of such a comprehensive surveillance strategy and eradication

programme approach would require the partnership of multiple organizations. Most importantly, commitment and buy-in at the country level would be required to make the strategy possible. While external funders could provide additional support, without consensus among partners for a robust programme, the approach would not be sustainable.

3.2b. Genetic epidemiology and disease surveillance for outbreaks: Ebola

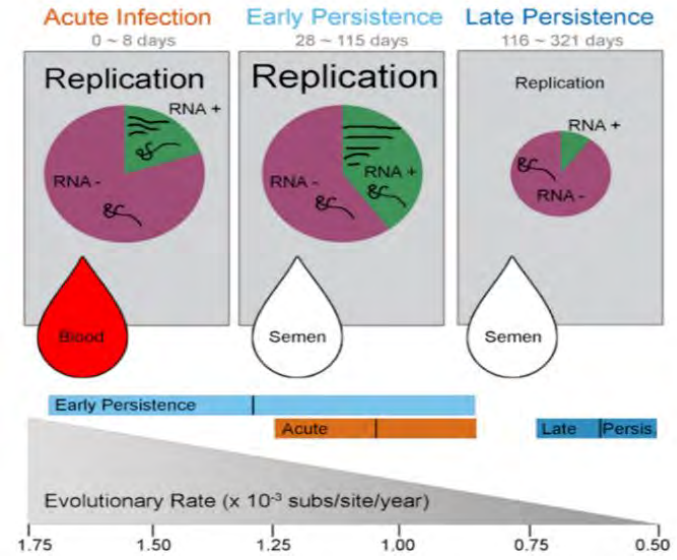
Presenter: Mark Perkins

The accessibility of molecular tools to support outbreak investigations and emergency situations has increased dramatically due to declining costs and increased ease of use, including the recent dramatic simplification of sequencing platforms. In this context, genetic epidemiology can help to supplement and fill gaps where conventional epidemiological methods have failed. Particularly in the current outbreak of Ebola in the Democratic Republic of the Congo (DRC), the utility and depth of information obtained through conventional epidemiology have been hampered by social issues within the community. Inadequate data yield an unclear or incomplete picture of the chains of transmission, which subsequently impacts outbreak management. Other key areas where flaws can exist in traditional methods include the over-/under-reporting of clinical cases, imperfect sensitivity or specificity of diagnostic assays, avoidance of health care facilities by at-risk groups or infected patients, misjudged or no information on close contacts, and inaccurate assumptions regarding the transmission source.

Incorporating sequencing routinely into Ebola outbreak management presents opportunities to improve accuracy in understanding the transmission and spread of the pathogen within the population. For zoonotic pathogens such as Ebola, it is important to distinguish between single introductions versus multiple transmission chains from animal reservoirs linked to human cases. Genetic markers can also identify transmission between individuals within the community, identify nosocomial spread and aid in the detection of infection control failures in health facilities, for example, transmission due to reused needles in pharmacies. The data can then be used to support the implementation of specific control measures to prevent human–human transmission or introductions from animal reservoirs by minimizing the risks of exposure related to clinical or community practices or the environment.

Another key utility of genetic data is to identify novel mechanisms of transmission, for example, through sexual contact or breastfeeding (Fig. 3). Additionally, using specific mathematical algorithms to analyse metagenomic and sequencing data can identify how many transmission chains have been missed, and help to estimate the true size and scope of an outbreak. This information can then be applied to decision-making for improving the surveillance system and expanding or targeting control measures in a given area. Genomic data can also reveal threats to the current medical countermeasures, such as mutations in PCR primer/probe sites, which could prevent the detection of the disease.

Fig. 3. Evolutionary rate of virus by stage of infection



While there are many opportunities and applications for genomic data to support outbreak management, as has been evident in the case of Ebola, significant challenges remain. Communication and information sharing, particularly in low-resource settings, can be critical. A platform or database would be needed to improve monitoring of traditional epidemiologic data and to ensure appropriate integration of the supportive information that genetic epidemiology data could provide. Furthermore, despite clear use cases for genomic data in decision-making, there has been no strategic change in the overall outbreak response to Ebola to make data use more systematic and streamlined. It is important to note that security issues in outbreak and crisis situations play a significant role in the ability to roll out molecular-based surveillance approaches. Even when capabilities exist in an area, the response can be thwarted by security and access concerns, minimizing the ability to implement a robust lab-based component to outbreak control. For example, in the current outbreak in the DRC, compared to the West African Ebola epidemic or previous epidemics in the DRC, serious security issues have prevented access to the geographical area of the outbreak. This situation has created major difficulties in managing the outbreak, despite past experience, collaboration and training in effective Ebola response using genomic epidemiology. As a result, minimal sequencing has been done, which has minimized the availability of statistical predictions and response algorithms to support decision-making. While some sequencing data are available, accurate assumptions cannot be made for Bayesian analyses because meta-data are lacking, rendering the sequencing data useless in that context.

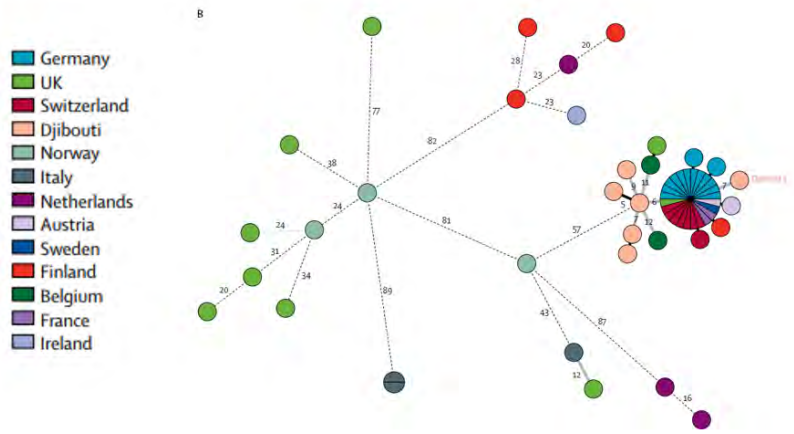
3.2c. Genetic epidemiology and disease surveillance for ongoing transmission: TB

Presenter: Anna Dean

For pathogens with ongoing transmission and varying burden around the globe, genetic epidemiology can support surveillance efforts to understand disease trends, changes in transmission over time, and threats to countermeasures such as emergence of drug resistance. The Global Project on Anti-TB Drug Resistance Surveillance, hosted by WHO, was initiated in 1994 and has since become the oldest and largest antimicrobial resistance project. The project estimates prevalence of drug resistance among people with TB, captures trends, and guides resource allocation, planning and policy development. Through its network of supranational TB reference laboratories, the project integrates whole genome sequencing (WGS) to conduct global surveillance and monitor trends in drug resistance. In high-income settings, WGS is being increasingly incorporated into investigations of cases of multidrug-resistant (MDR-) TB, such as in a recent cross-border outbreak of MDR-TB in Europe among migrants from the Horn of Africa (Fig. 4). This investigation was possible because of the level of capacity and collaboration present throughout the region. However, the capacity for routine surveillance varies.

Paradoxically, the countries with the highest disease burden are often the ones with the lowest capacity; they must rely on nationally representative surveys conducted periodically to estimate disease burden. Low capacity for continuous surveillance impacts the timeliness and availability of data for decision-making on drug resistance patterns or transmission, and for understanding the true prevalence of disease.

Fig. 4. Cross-border outbreak of MDR-TB *(2)*



58 patients with related MDR-TB strains

At country level, data generated from the project are used to identify local and regional needs for medicines and resources and can inform programmatic decisions based on the resistance patterns identified. Countries can use the data to set national targets for rifampicin-resistant TB through case finding, calculate second-line drug needs, assess the feasibility of new treatment regimens and guide national diagnostic algorithms. Conventional diagnostic methods followed by phenotypic drug susceptibility testing on culture isolates takes weeks to months. Rapid molecular testing using the GeneXpert platform or other tools presents a significant advantage in terms of the timeliness of results at the peripheral level, although this is currently limited to rifampicin only. In addition to the decreased length of time required for accurate results, sequencing also provides information on transmission chains and case clusters.

There are significant advantages associated with the incorporation of sequencing data into national drug resistance surveys. In addition to improved accuracy and reliability of testing for drug resistance patterns, these data allow for the assessment of the feasibility of new drug regimens and decision-making to guide programme efforts. Unfortunately, in most places, sequencing is not a local capability and must be outsourced to laboratories within the network. Capacity-building is required to make sequencing sustainable in regions with the highest density of transmission. Other considerations for scaling up sequencing in TB surveillance include the technological requirements (e.g., sample types: culture needed for WGS compared to either culture or sputum for targeted gene sequencing), logistics for sample transport systems, biosafety, and expertise required. There also needs to be increased local capacity for data storage, standardization of data, data reporting and interpretation, and cost–benefit analysis for implementation of an ongoing surveillance programme.

Incremental next steps that can improve access to next-generation sequencing technologies will be implemented with WHO support. WHO released policy guidance in 2018 on standardizing the approaches to conducting sequencing and interpreting results through standardized pipelines *(3)*. WHO has also developed and houses a multi-country database for countries to directly send sequencing data where the information can be safeguarded. The database currently contains population-representative isolates from 13 countries, with approximately 12 000 isolates in total. It will serve as a repository that can be used to support WHO analyses and data aggregation by region

and to improve understanding of disease trends globally. To minimize concerns over data ownership and management by participating countries, the database will be closed and not available for manipulation and use externally. Data sharing depends on the country: While the majority of countries are willing to share data and send samples to partners for analysis, other countries prefer to rely on local data analysis and choose not to outsource molecular work to regional partners or share data that are generated locally.

WHO is also playing a role in promoting regional and country capacity for local sequencing efforts; for example, in Africa, there are efforts underway to increase capacity beyond the laboratories in South Africa, Benin and Uganda. WHO has also produced policy guidance and translation for action. At the country level, interest in incorporating sequencing efforts into national programmes may be shaped by different priorities. For example, some countries may be more interested in support for individual clinical case management, although interpreting the results is not straightforward. In the context of national surveys, sequencing data cannot be used for clinical decision-making, since data generation is too slow to support case management and such surveys are only conducted periodically (approximately every five years). However, data are being used to support revisions and adaptations of national diagnostic algorithms. For policy guidance, collaboration with the WHO Global TB Programme has supported guideline development and provided lessons learned to other diseases such as HIV that are further behind in their capacity for global drug resistance surveillance. Overall, the WHO Global TB Programme will continue to support national TB programmes and help meet their needs to establish quality surveillance programmes.

3.2d. Discussion: Key considerations in application of genetic epidemiology

There are several key considerations in the application of genetic epidemiology to the surveillance of polio, Ebola and TB. These include the type of pathogen, mode of transmission, and current situation for management and control within the population. Lessons learned from the use cases in ongoing surveillance programmes, eradication efforts and outbreak responses can be used to inform next steps in malaria genetic epidemiology efforts. Use cases that have been effective in supporting surveillance include 1) understanding transmission links and 2) identifying missed transmission chains. These use cases have been significant in gauging the intensity of the disease event and prevalence of disease and helping to target prevention and control strategies based on the populations affected and at risk.

Despite the clear potential for use of genomic data in polio, TB and Ebola, key challenges were also highlighted across other diseases in terms of i) cost, ii) capacity and iii) data generation, sharing and use of information. Countries have varying capabilities and capacity for local genetic epidemiology methods and interpretation of data. In addition, depending on health system structures, facility set-up and maintenance can be difficult. Particularly in crisis settings, security is a major concern, presenting additional needs for maintaining adequate biosecurity around facilities, equipment and samples. For example, in the current Ebola epidemic, despite the presence of local facilities and technical capabilities, armed groups have targeted health facilities and laboratories, endangering the safety and security measures needed to keep the facilities open. In cases where network availability can support implementation of a genetic epidemiology programme, there are still local and national network needs for managing quality assurance and ensuring consistency in the logistics for sample movement, storage and testing results. In addition to data reliability, data ownership and reluctance to share data present further data challenges. In outbreak settings such as Ebola, the WHO R&D blueprint on pathogen genetic sequencing data and code of conduct for open and timely sharing of data have proved useful. However, this has not been translated for ongoing surveillance and elimination programmes in order to provide guidance on consensual data sharing and use. Lessons learned from previous applications of genomic data and the key considerations for further use cases will prove fruitful in informing future scale-up efforts and the incorporation of such data into other disease control and elimination programmes.

3.3. Session 2: Overview: malaria parasite, anopheline gene flow, modelling

Opening remarks by facilitator, Dyann Wirth

Gene flow is a generic term that describes the spread of genetic material between populations and/or locations. For example, gene flow between two locations implies that there is migration between these two locations, whereas gene flow between two genetic subpopulations implies that there is interbreeding between the subpopulations. Understanding gene flow in malaria parasite populations has the potential to drive the implementation of surveillance strategies to control spread, monitor resistance and evaluate the effectiveness of interventions. Gene flow can be measured across the whole genome or at specific loci. Differences in the rate of gene flow are related to the mutation rate, which varies at different loci through recombination or evolutionary selective pressure. Measuring genome-wide gene flow can provide estimated rates of dispersal, migration and interbreeding, whereas locus-specific gene flow can estimate rates of spread of drug resistance, insecticide resistance, and gene drive.

3.3a. Tracking gene flow in malaria parasite populations

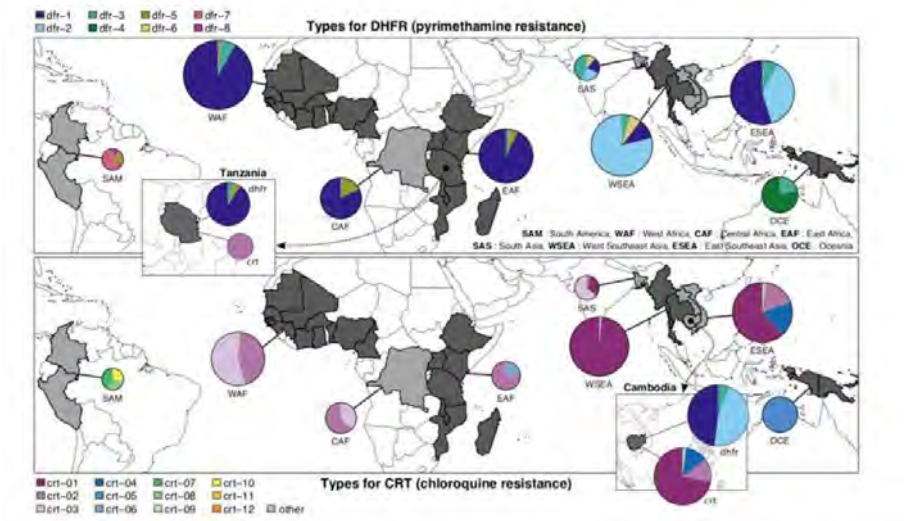
Presenter: Dominic Kwiatkowski

For malaria control, it is essential to distinguish between analytical use cases and operational use cases for understanding gene flow. The former involves understanding changes in epidemiology, whereas the latter is about applying genetic information to the decision-making process – a subtle but significant difference. Analytical use cases include efforts to understand changes in transmission intensity, identify hotspots, and determine rates and routes of transmission. By contrast, an operational use case of malaria genomic data would inform plans for elimination zones, containment strategies for multidrug resistance, or approaches to tackle the resurgence of malaria in an area. When establishing a genetic epidemiology surveillance system, it is important to consider the type of use cases that are anticipated in order to ensure that the appropriate methodology and approach are being used to generate the type of data that can support analytical and/or operational use. For *Plasmodium*, this can be particularly challenging. It is necessary to maintain centralized, open genome sequencing data in order to understand lineages and recent common ancestry. For example, if two parasites have the same sequence at a large haplotype locus (e.g., >30kb or ~2cM), this implies that they must have a recent common ancestor at that locus and are of the same lineage. It is also necessary to understand the type of genotyping technology needed to generate these data. In the context of the emergence of resistance in malaria vectors, surveillance programmes can use specific markers (e.g., SNP barcodes or known markers of drug resistance), amplicon sequencing (e.g., haplotypes and new mutations at known resistance loci), or genome sequencing (e.g., signals of recent selection due to new forms of resistance). Chromosomes in a eukaryotic parasite like *Plasmodium* undergo meiotic recombination with every sexual generation. Consequently, there is high variability in the genome such that two randomly sampled parasites are unlikely to have the same chromosomal haplotypes. Therefore, sequencing technology in a surveillance programme must meet certain requirements so that it can provide useful data for understanding malaria epidemiology and key elimination concerns, such as imported cases in elimination zones or the emergence of resistance markers in a region.

Keeping in mind the complexities of gene flow in malaria parasites at specific loci, in the use case of understanding resistance, it is important to note that most forms of drug and insecticide resistance have multiple lineages with different patterns of spread and that some lineages can spread more aggressively than others. For national malaria control programmes (NMCPs), epidemiological interests lie in what resistant lineages are present in the region and which ones are newly emerging. For example, to understand the spread of artemisinin resistance caused by *kelch13* mutations (KEL1) *(4–7)*, a tiered phase approach was implemented. Phase 1 investigated the emergence of KEL1 in different parts of South-East Asia with notable localized geographic distribution. Phase 2 then investigated the rapid expansion of a related group of parasites that shared a specific lineage of KEL1 and a specific

lineage of plasmepsin amplification (PLA1) that caused DHA-PPQ treatment failure in western Cambodia. The current phase 3 is investigating the KEL1/PLA1 co-lineage that has spread across the region and differentiated into sub-lineages that vary in geographical distribution and phenotype. While there are a number of resistant lineages, only certain lineages are sustained and continue to spread. MalariaGEN is producing global estimates of *Plasmodium falciparum* multidrug resistance based on genome sequencing of 7000+ samples to identify the most successful lineages of pyrimethamine resistance and chloroquine resistance. The project also promotes longitudinal genomic surveillance to support further analytical and eventual operational use cases for sequencing data (Fig. 5).

Fig. 5. Winning lineages of pyrimethamine resistance and chloroquine resistance based on genome sequencing of 7000 samples



A key application for understanding the gene flow of resistance in *P. falciparum* includes tracking outbreaks to determine the development and movement of resistant lineages as opposed to simply identifying whether resistance is present or not. Considering the diversity in the global population compared to local parasite populations, comparative analysis of point mutations at specific loci are not informative about parasite migration. Rather, understanding migration scenarios requires the ability to discern between external introduction, parasite movement between areas, and mixing or interbreeding in a given area. This analytical use case then gives way to operational applications in decision-making. Maintenance of shared resources, open data sharing, and capacity for data generation and sample testing are necessary to advance the field towards effective use cases for decision-making, programmatic and intervention impact, and guidance for resource allocation. Moreover, a framework for understanding the connection between genomic data and their application to interventions or policy-making needs to be clearly defined in order to facilitate the use of data in decision-making. For example, genotypic signals can be informative for understanding or expecting a phenotype in a region. These data can then inform survey strategies and further data collection to confirm genetic implications, thus providing stronger evidence to support decision-making based on genetic epidemiological information. However, this flow and approach to programmatic work would imply a significant change in the general framework for interpreting specific data to inform decision-making for malaria.

3.3b. Tracking gene flow in anopheline populations

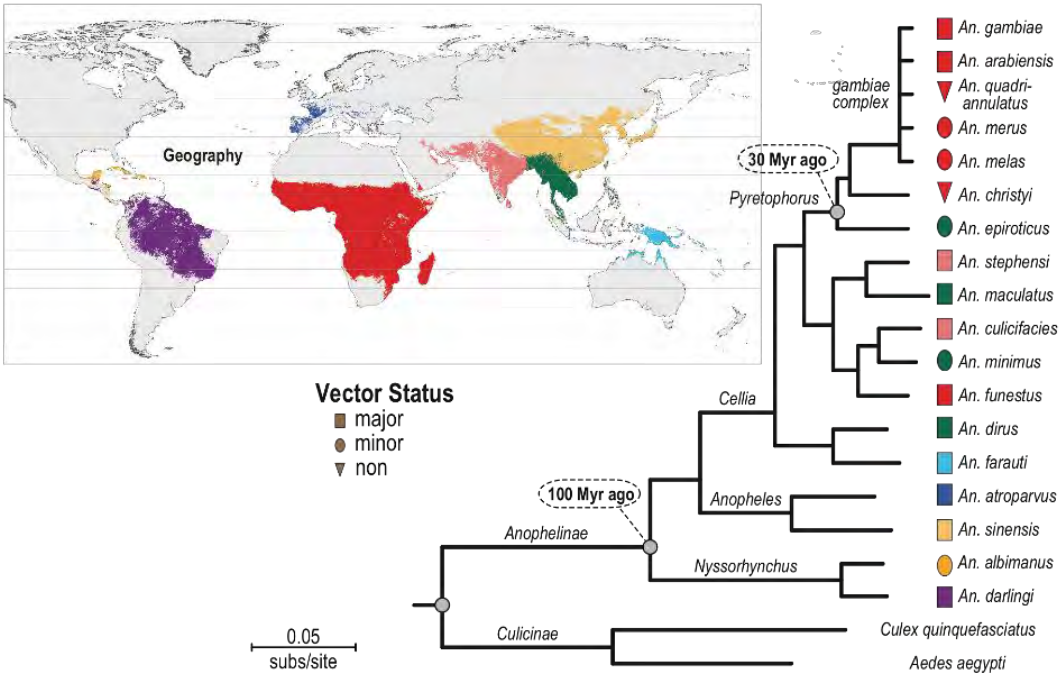
Presenter: Daniel Neafsey

Understanding and tracking gene flow in anopheline mosquitoes is complex given the amount of genetic diversity that exists within mosquito populations. There has been a long-standing need to

improve integration between the field of molecular and medical entomology and the field of public health, along with a long history of understanding the underlying genotypes that are linked to phenotypes observed. Chemosensation, which governs many phenotypes including host feeding preference, and mosquito immunity to infectious microbes (including human *Plasmodium* parasites) are among the most rapidly evolving traits in mosquitoes. These traits contribute to the heterogeneity of mosquito populations and changes in complex traits such as vectorial capacity over short evolutionary periods. Vectorial capacity is dependent on multiple factors that vary among species, such as chemoreception, circadian rhythm, immunity to the parasite, insecticide resistance, reproduction, larval development habitat, and aridity tolerance, among others. Local vectorial capacity is therefore a function of species composition, and changes in this composition can impact malaria transmission. Comparative genomics can be applied to understand differences in vectorial capacity and their impact on malaria transmission (Fig. 6).

A multi-locus approach is needed to understand anopheline gene flow because inversions, divergence and introgressions can occur, making a single-marker approach less than informative. Patterns in gene flow and species divergence are not uniform and can occur at different locations and rates along chromosomes. Such patterns can be missed if using a single-marker approach. Specific mutations can either lead to gene flow in populations or suppress it. Inversions are linked to niche specialization and lead to suppressed recombination and subsequent suppression of gene flow. Introgressions or interspecific genetic exchange, on the other hand, can lead to rapid changes in vectorial capacity. It is also important to note that there are still undiscovered species, cryptic species and aspects of vector ecology that are not fully understood. Consequently, attributes of unknown vector parasite interactions and vectorial capacity that have yet to be measured can have potential impacts on key concerns for malaria control, such as resistance patterns and intervention impact.

Fig. 6. Mosquito comparative genomics to understand differences in vectorial capacity *(8)*



Among other considerations in control programmes, new technological approaches such as gene drive and the use of genetically modified mosquitoes leave unknowns related to impact on the population dynamics of natural mosquito populations. For example, forced selection and gene drive may allow

for introgression that may not otherwise occur. Sporadic hybridization events are also possible, but not sufficiently accounted for in current uses and studies related to gene drive. A whole genome strategy may be useful, namely in discerning cryptic barriers to gene flow and generating evidence on the emergence of hybrid mosquito populations. In addition, identification of common lineages and ancestors can also foster better understanding of migration patterns of mosquito populations and connectivity in a region.

In general, there is a need to improve surveillance of shifts in populations, understand rates of migration and insecticide resistance patterns, and consider the necessary studies and scales to determine the effects and impacts of gene drive. Opportunities for applying genomic tools to further understand mosquito populations and their movements or introductions to new areas, with consideration for environmental changes and emerging issues such as climate change, are also important. A WGS strategy for local vector and non-vector species, identification of key local markers for taxonomy and insecticide resistance, and large sample collections for genotyping can help further strategies for malaria control and elimination.

3.3c. Discussion

In identifying use cases for malaria genetic epidemiology, the key is in discerning where the evidence is strong enough to consider policy development, and where additional information is needed to strategically develop research guidance that could later inform policy and operational use. In reviewing the use cases based on gene flow, a major concern is the exclusion of genomics in diagnostics from the Technical Consultation. Clinical applications of genomics and the impact of *pfhrp2/3* deletions on diagnosis need to be addressed. There are concerns that, in the future, rapid diagnostic tests (RDTs) will no longer be viable in Africa and therefore genomics and proteomics could be used to identify new markers for the development of new diagnostic tests, in particular rapid diagnostics that can be used in the field. For some, the advancements in genomics and the available technology for molecular diagnostics and speciation of parasites make identifying use cases for clinical applications ideal. However, development of new tools such as rapid diagnostics and identification of new markers are outside the scope of the current consultation. Rather, it is important to distinguish between operational and analytical use cases in surveillance or elimination certification contexts and identify use cases that are more actionable in a research space, such as the use of genomic data to drive identification of new resistance markers.

With expectations of operational use cases in genetic epidemiology, issues surrounding the generation of genomic data and subsequent data storage and data sharing need to be considered. Data guidelines and agreements are needed in a normative context in which WHO can offer support for longitudinal data generation in order to strengthen use cases with conceptual evidence that lack data for comparison and evidence in the field. In this respect, a repository or data storage platform that can support metadata aggregation during data collection could enhance data interpretation and integration for the decision-making process. This would also require capacity for management of big data and the possibility of data sharing within a region. Assessing what health system structures exist and what NMCP capacity is available will inform the development of or recommendations for any data storage platform. It is important to ensure that such a platform is not only functional now but can also be adapted to future needs. Knowing what decisions the data will support can inform system development so that relevant data are generated and stored, with the awareness that NMCPs and policy-makers may have different data needs to support alternative decision-making processes.

Other use cases considered integral to understanding malaria transmission dynamics and informing elimination programmes include application to questions on importation. Currently, determining importation is often reliant on travel history and conventional epidemiology methods. Genomics can offer improvements in the process and more accurate data. When conventional metrics are used, despite their utility, there are often gaps; such gaps have been evident in Ebola outbreak management for example, and are even more complex in low transmission density settings for malaria. Applying

genetic epidemiology methods using geospatial frameworks alongside genomic data on transmission chains can provide further inference into population-level transmission that may otherwise be incomplete.

From a funding perspective, understanding transmission dynamics on a more refined level and the application of genomic data in decision-making could help to elicit financial support for resources required to implement the use of genomic data in malaria control in both high-burden and elimination settings. Understanding current needs and projecting future needs will help to inform areas for capacity-building, appropriate settings for genetic epidemiology use, and generation of rich data that can support future policy recommendations and programmatic decisions.

3.4. Session 3: Parasite gene flow and the spread of drug resistance – setting the scene

Opening remarks by facilitator, Pascal Ringwald

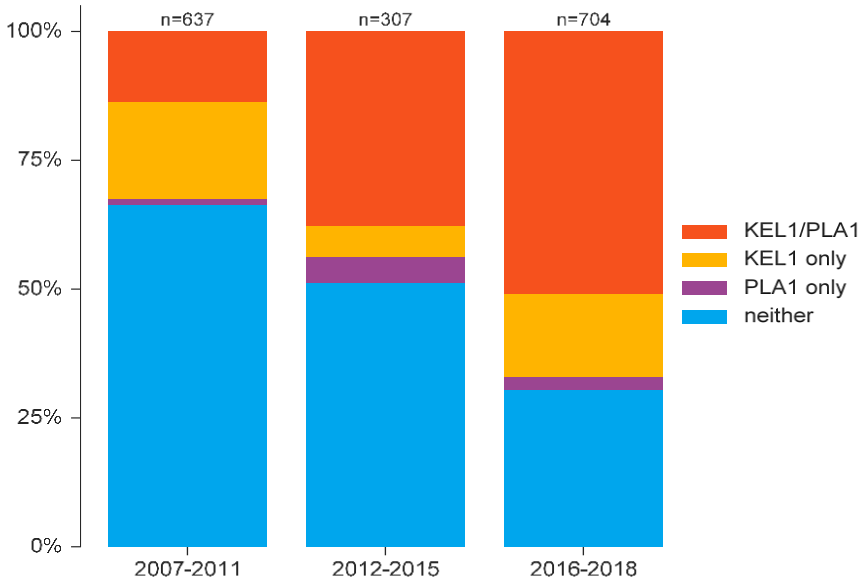
An important aspect of understanding the spread of drug resistance in the context of parasite gene flow is in determining the geographic origin of resistant parasites. It is necessary to discern if resistance emerged locally, or if a resistance gene was imported and subsequently disseminated within the local parasite population, thereby becoming established in a given area. There are several approaches to determining natural emergence or introduction when tracking resistance spread. However, depending on the genomic technology applied, results may be reliable and accurate, or leave questions and uncertainties. In terms of tracking drug resistance as a use case for malaria genetic epidemiology, the key questions are related to the minimum and optimal information requirements needed, and how to ensure precision of methods when determining the geographical origin of resistance genes.

3.4a. Tracking the spread of drug resistance using gene flow data

Presenter: Olivo Mioto

In South-East Asia, the GenRe-Mekong project has demonstrated the use of gene flow to track the spread of drug resistance. By integrating genotyping of dried blood spot samples and reporting on marker genotypes into routine NMCP activities, it has been possible to determine which use cases have some utility and identify gaps in how data have been translated. Understanding the gene flow of resistance in the region is crucial, as there has likely been a combination of a spill over event and selective pressure, but this is poorly understood. The project has shown clear geographic differences in resistance patterns for artemisinin and piperaquine, and pressure in certain areas that has aggressively contributed to the parasite population being replaced by introduced parasites (Fig. 7). The spread of the introduced parasites has been linked to gene acquisition, which has then facilitated spread to surrounding countries. Low resolution data from 101 single nucleotide polymorphism (SNP) barcodes have been used to discover the population structure and the movement of the resistance genes. However, even in areas where the KEL1/PLA1 co-lineage has excelled in terms of spread, there are still nuances in the population structure and variation in distribution that need to be understood *(9)*. It is also important to understand regional differences within a country because, due to differences in local resistance patterns, genomic data from one region may not appropriately inform decision-making in another area.

Fig. 7. DHA-PPQ resistance in the Greater Mekong Subregion *(9)*



In determining appropriate methods for understanding the emergence of resistant parasite populations, it is essential to have baseline whole genome data available for comparison. For example, in Papua New Guinea (PNG), where there was limited access to regional parasite whole genome data for comparison, a reference database of sequences from other South-East Asian countries was a critical aspect in accurately identifying the origin of the resistance patterns observed. Initial investigations compared microsatellite/SNP genotyping from parasites isolated from PNG data with resistant reference parasites and local parasites. In investigating CY580 mutant parasites to determine natural emergence versus importation from South-East Asia, identity by descent (IBD) analyses could not confirm where the parasites were from. There was confirmation of similar haplotypes corresponding to parasites from the South-East Asia region, and confirmation that there was no recent introduction. At the same time, there was some suggestion that there may have been early spread from South-East Asia, which allowed for similarities in the patterns of resistance emergence. Multiple lines of evidence using various genomic methods were needed to identify these different attributes and understand the parasite population, especially since current tools for IBD are not entirely clear when reconstructing the origin of resistance alleles. Most importantly, a large database of parasites from multiple regions needs to be available for comparison. This will allow for improved efficiency in comparisons across regions. While this approach is useful for understanding parasite origin, there are several limitations and results are not definitive. Extensive whole genome surveillance would be key to more reliable analyses, especially as emergence and importation may be more complex than expected.

3.4b. Genomic structure, diversity and migration of *P. falciparum* in South-East Asia

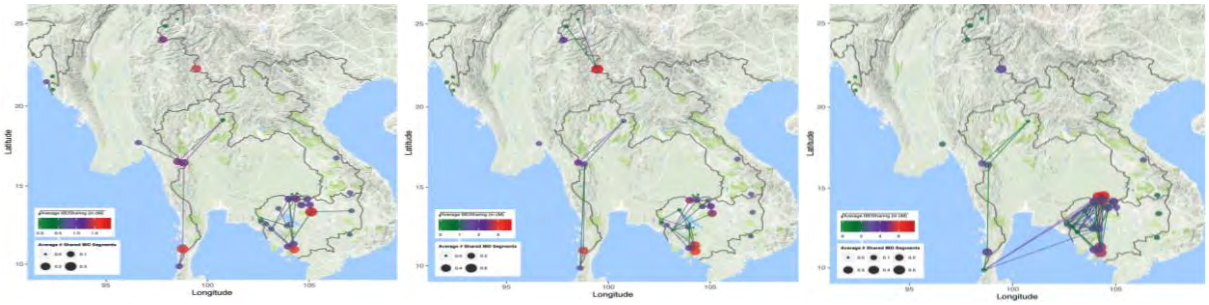
Presenter: Shannon Takala-Harrison

In areas such as South-East Asia, where there have been ongoing efforts to eliminate malaria in the Greater Mekong Subregion, it is important to further understand the factors driving malaria risk in order to prioritize resources and optimize elimination strategies. Estimates of parasite migration are important in stratifying malaria risk, providing information about where parasites are moving or where there are barriers to parasite movement. Parasite migration has often been inferred based on human movement (regardless of infection status) from areas of high malaria prevalence. These studies are informative, but do not directly measure parasite migration. Thus, use of malaria parasite genomic data to understand parasite population demography can augment studies of human movement to

understand parasite migration. In efforts to understand genomic structure, diversity and migration of *P. falciparum* in South-East Asia, different approaches have been applied.

A recent study aimed at mapping patterns of parasite population structure and inferring migration patterns using IBD analyses to determine the age of shared ancestry among sample isolates and estimate regional relatedness *(10)*. The study identified parasite genetic population substructure at a district level, based on shared IBD genomic segments. Parasites sampled from sites along the China–Myanmar border and in Bangladesh were relatively isolated from parasite populations in other regions of the Greater Mekong Subregion (Fig. 8), showing low genetic relatedness with parasites from other study sites based on IBD sharing. In addition, IBD estimates indicated connectivity between parasite populations along the Thailand–Myanmar border and within northern, western and southern Cambodia, but very little connectivity between parasite populations on the Thailand–Myanmar border and Thailand–Cambodia border, consistent with other studies that have indicated genetic differences between parasites in the western and eastern Greater Mekong Subregion. This study was also able to explore directional parasite migration based on admixture estimates, as well as likely drivers of increased IBD sharing in recent timeframes among parasites sampled in Cambodia, such as the selection and spread of multidrug resistance. While the IBD analysis in this context proved useful for understanding the parasite population structure in this geographic region, this approach did not explicitly model the spatial structure of the genomic data.

Fig. 8. Regional relatedness between parasites in South-East Asia *(10)*



A tool called estimated effective migration surfaces (EEMS) can be used to visualize spatial patterns in the data, allowing for visualization of geographic regions of more or less effective parasite migration for a given geographic distance between different sampling locations. EEMS does not currently allow for inference of directionality of migration. Additionally, genetic distance metrics need to be modified in order to better reflect more recent migration patterns and inform decision-making for operational use. The tool and approach can aid in understanding parasite population structure and migration and could potentially identify geographic units for interventions. However, the tool will require optimization to make it more spatially explicit to estimate local versus long-range migration patterns, and account for the impact of sample size and grid density to ensure accuracy in analyses.

3.4c. Discussion

In discussing approaches to understanding gene flow of resistance by geographic origin, there was a consensus that IBD analysis is a useful research tool but is not practical for NMCPs in its current state. The methodological approach would need to be distilled down to something that could be applied as a use case for control programmes in the future. For this to be feasible, it is clear that more rapid techniques and standardized markers would be needed at a minimum. In addition, data generation using a WGS approach across varying parasite populations from different countries and from geographical areas within the same country would be required for comparison. Considering the time needed to collect these data, a database would also be needed to store information over time. Some concluded that the information would be useful to uncover drug resistance patterns across the

genome, instead of just at specific resistance foci, but that proof of concept is still needed in areas of mixed infection. Moreover, at a global level, although these data could be used to answer questions on whether certain drug-resistant parasite lineages are spreading between countries or regions, the approach is not timely and cannot be used in its current state to make policy decisions on treatment. Confirming population structures and understanding migration are currently research priorities; yet, these approaches do not provide conclusive evidence that can be used for programmatic action at this time. More research is therefore needed before IBD analysis can be used in operational use case scenarios.

3.5. Session 4: Parasite and mosquito genetics to understand transmission intensity – setting the scene

Opening remarks by facilitator, Jan Kolaczinski

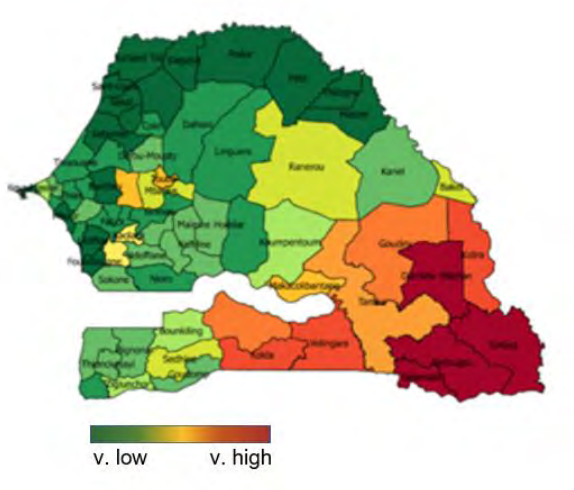
When it comes to understanding gene flow in parasites and mosquitoes as it relates to transmission intensity, there are priority questions in malaria surveillance, vector surveillance, insecticide resistance management and evaluation of new vector control tools. For surveillance, it is necessary to better understand importation risk and receptivity, and changes in transmission intensity over space and time. Within the vector population, drivers of population change, spatial and temporal variations in the patterns of resistance, and adequate methods and sampling strategies for measuring such changes are important considerations. In addition, in terms of novel control tools, such as gene drive and genetically modified mosquitoes, genomics has the potential to contribute to field evaluations designed to assess the effects on local populations and selection for resistance to these new tools. WHO requires high-quality evidence to support the development of guidelines and practical manuals to support implementation of surveillance activities and deployment of interventions by NMCPs.

3.5a. Parasites: tracking gene flow and its relevance to transmission intensity

Presenter: Daouda Ndiaye

The elimination programme in Senegal has successfully applied genetic epidemiology in the control of malaria as a result of good local capacity for implementation, operational research partnerships and collaboration to monitor and evaluate elimination progress. Community engagement has aided in the acceptance of genetic epidemiology as a means to monitor progress towards elimination and support outbreak investigations. In Senegal, interventions are stratified according to transmission intensity, and use cases are targeted towards answering key questions for malaria control and elimination in order to measure intervention progress and impact (Fig. 9).

Fig. 9. Stratification of malaria incidence in Senegal



One key area where genetic epidemiology has been applied is in detecting persistent local transmission in low transmission areas and determining changes in parasite populations in high transmission areas. In areas with very low incidence (<1/1000) and presumed no local transmission, the identification of identical parasites persisting across multiple transmission seasons suggests that local transmission is ongoing despite low rates. Genomics can confirm that there has been persistence of the same parasite population over time rather than importation or new emergence of another parasite population from a different area. This information can then contribute to progress in malaria control in the area and intervention success. This use case of understanding parasite composition nationally and regionally can then inform elimination progress. Similarly, in detecting changes in high transmission areas, increasing parasite relatedness can be considered a possible early indicator of intervention impact. Alternatively, in an outbreak scenario in which parasites are confirmed in a location where transmission should not be occurring, the use case of understanding transmission foci can allow for case cluster investigations and confirmation of elimination success by characterizing an introduced parasite.

In Senegal, where programmatic application of genetic epidemiology methods has been established, there is an opportunity to scale up surveillance approaches. The current way forward is to scale up the capacity to apply tools in use case scenarios with past success and continue to integrate data from routine surveillance activities for both parasite and vector populations, monitor resistance, and conduct spatial risk mapping. Continued use of traditional epidemiologic data alongside supplementary genomic data to provide additional evidence and precision in complex transmission scenarios will support programme decisions and exemplify the application of genetic epidemiology at the NMCP level.

3.5b. Tracking gene flow and its relevance to insecticide resistance – the example of *Anopheles gambiae*

Presenter: Alistair Miles

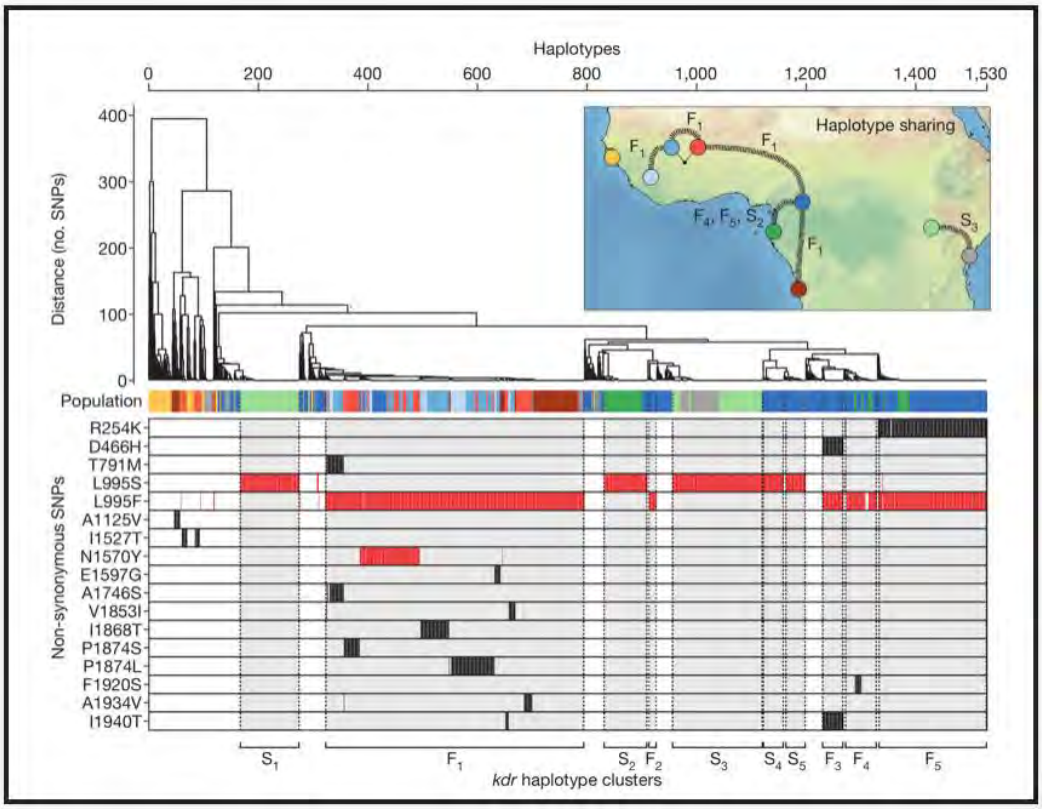
In determining use cases relevant to tracking gene flow in vector populations for insecticide resistance, the irony is that decision-making consistently excludes molecular data that could support decisions that are inherently molecular. Practical use cases for incorporating genetic epidemiology data include assisting decision-making on whether to procure next-generation long-lasting insecticidal nets (LLINs) or whether to deploy indoor residual spraying (IRS) and facilitating the coordination of cross-border resistance issues. Many of NMCPs' key questions on where and what resources are needed often require some genomic data, especially if the decisions made are to be truly informed by the transmission situation.

As previously mentioned, access to WGS data representative of a region can facilitate research and surveillance for improved understanding of the gene flow that is occurring. For mosquito populations, efforts are underway through the *Anopheles gambiae* 1000 Genomes Project (Ag1000G) to create an open database of anopheline genomes in order to aid investigation of genetic variation and evolution in natural mosquito populations. The project employs a three-phased approach to collect data on *An. coluzzi*, *An. gambiae* and *An. arabiensis* across a broad geographic range of up to 18 countries. These efforts have provided insight into the genetic variation that exists in areas of malaria transmission.

When investigating gene flow, in addition to understanding flow between species and locations, it is also important to understand changes across population generations. Certain genes may be under stronger selection, for example, and increase in frequency within the population. Understanding these changes can aid in making predictions for future generations of mosquitoes and identifying genetic indicators or markers of resistance patterns that may emerge. Specifically, in understanding selection of resistance genes, evidence of selective emergence related to a particular driver or isolated instances across populations can also provide the basis to infer influences on gene flow.

Two examples of selection and spread of resistance genes include pyrethroid target-site resistance and pyrethroid metabolic resistance. With pyrethroid target-site resistance, the spread of "knock-down resistance" (*kdr*) mutations in the voltage-gated sodium channel gene (*Vgsc*) was of concern. Two known *kdr* mutations were in circulation and there were questions as to whether the mutations were spreading and where gene flow was occurring. Gene flow could be inferred by identifying the same *kdr* haplotype in two different locations using over 1700 biallelic SNPs from all mutations within the *Vgsc* gene (Fig. 10).

Fig. 10. *kdr* haplotype clusters



With pyrethroid metabolic resistance, it was important to determine the spread of copy number variations in cytochrome P450 genes, because increased gene copy number implies increased expression and increased expression implies pyrethroid resistance. There are multiple P450 genes in the genome; for example, *Cyp6p/aa* and *Cyp9k1* are two loci where gene amplifications are common. Gene flow could then be inferred by confirming duplicate resistance loci across different populations. Interestingly, it is clear that gene flow is occurring and that different patterns of spread exist. While multiple independent events drive resistance, some spread, whereas others remain localized. The key lies in using this information to address strategic programmatic questions such as where best to deploy piperonyl butoxide (PBO) LLINs based on the evidence of resistance gene flow occurring within a region, while also considering factors related to cost and logistics.

While there have been many advances in understanding particular vector species, there are still gaps in the broader understanding of mosquito populations. There are still unknowns over what potential vector species may be present that may be contributing to transmission. These species may even be contributing to selection pressure for genes and influencing gene flow without any notable data or information to reveal the full scope of what is happening. Efforts such as Ag1000G can further support by scaling up genome sequencing of vector populations, increasing geographical coverage, conducting regular seasonal sampling in different areas and including other vector species. In addition, there is a

need to bridge the gap between research and national programmes to begin to investigate and apply gene flow information in more analytical and operational use cases.

3.5c. Discussion

Despite clear examples of genomic epidemiology use cases in understanding intervention impact, surveillance of resistance, and progress in control programmes, there are still some questions as to what types of data are truly needed to inform decisions and support NMCPs. Rather than isolated use cases where genomic data have been deemed useful in verifying conventional epidemiology information or in supporting a single procurement decision, there is a need to determine the utility of robust genomic data generation for supporting strategic decision-making over time and across various transmission scenarios. For example, in discussing the use case of procuring PBO LLINs, a combination of factors such as cost and logistics would need to be considered in decision-making, not solely the understanding or awareness that there may be gene flow occurring in the locale that is contributing to the presence of resistance patterns. There are concerns related to the implementation of new tools in terms of further driving gene flow and selection of future resistant mosquito populations. Could rolling out new chemicals to address resistance lead to similar issues as seen with the poor use of new drugs and antibiotics that has contributed to the emergence of antimicrobial resistance? With regard to using genetic epidemiology to provide information on decreased parasite population diversity and reduced intermixing by region, what programmatic changes or best practices could be identified to support use of this information for implementation in other geographical areas? There is still a need to simplify tools and address limitations in the timeliness of data collection so that the data generated remain relevant while supporting decision-making. Additionally, the identification of use cases in understanding parasite and vector population genetics for malaria surveillance is being carried out disparately. If new resources and tools are to be introduced, there needs to be increased coordination between parasite and vector control applications in order to ensure strategic implementation of tools by NMCPs.

3.6. Session 5: Parasite and anopheline gene flows to understand importation and identify foci of transmission

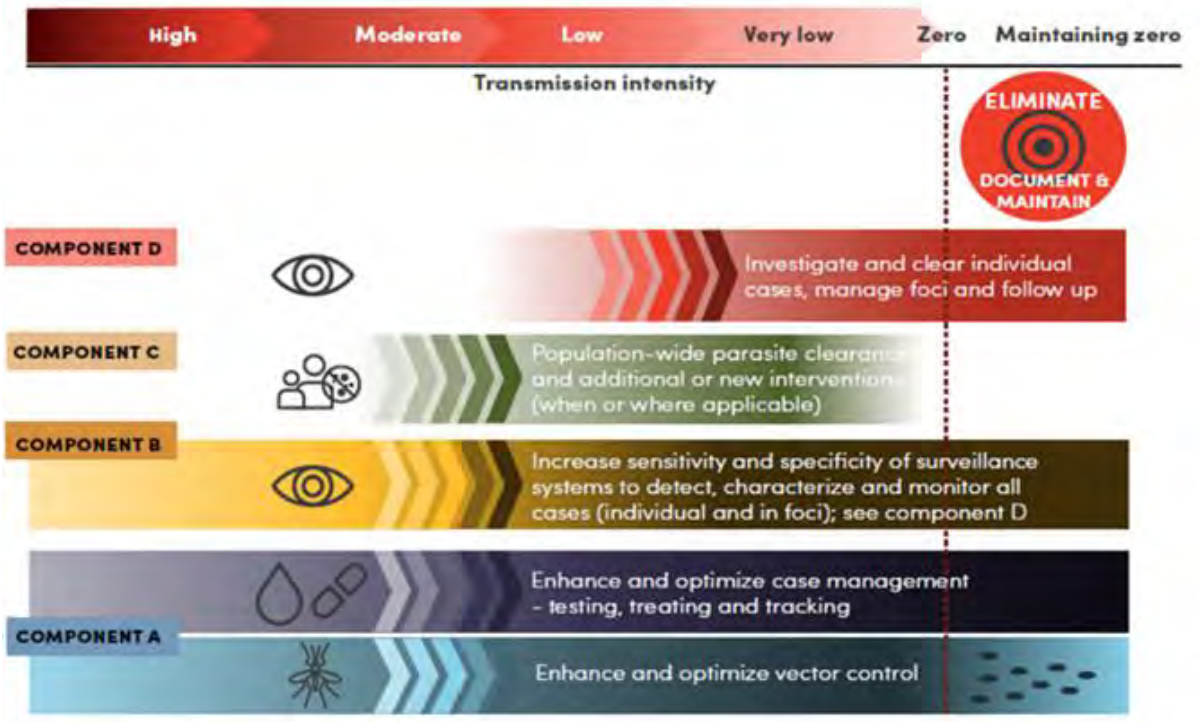
Opening remarks by facilitator, Kimberly Lindblade

Genetic epidemiology can play an important but varying role in the control of malaria across the continuum from high to zero transmission (Fig. 11) *(11).*[3] In elimination settings, countries experience many years of low-level transmission before reaching and maintaining zero cases. In low transmission settings, strong passive surveillance, comprehensive case investigations that elucidate the likely location of infection, active case detection, and targeted interventions in foci of active transmission are important. In areas with high malariogenic potential, preventing re-establishment is essential. An identified need and opportunity for genetic epidemiology is in providing evidence to support the correct classification of cases as imported, introduced or indigenous, particularly in the absence of epidemiologic data. In “getting to zero”, it is necessary to understand whether resurgences are due to poor case detection and surveillance, or whether persistent transmission is a result of repeated importations or indigenous transmission. Adequate spatial resolution could improve our understanding of cross-border transmission and case origin, thus also supporting efforts to prevent re-establishment. Genomic data are likely to be useful in improving our understanding of the transmission dynamics in eliminating countries. However, their operational utility will depend on the

[3] WHO has guidance on the tools, activities and strategies required to achieve malaria elimination and prevent re-establishment of transmission in countries, regardless of where they lie across the spectrum of transmission intensity. The framework informs national malaria elimination strategic plans and should be adapted to local contexts. Download the framework at: http://apps.who.int/iris/bitstream/10665/254761/1/9789241511988-eng.pdf

quality of surveillance and epidemiologic data collection, the availability of recent genomic data, data accuracy and the time it takes to generate the data.

Fig. 11. WHO Framework for malaria elimination 2017



3.6a. Use of genetic evidence in the Pan American Health Organization (PAHO)

Presenter: Kumar V. Udhayakumar

The application of molecular tools to support programmes in post-elimination settings in the PAHO region requires a database of parasite genomes to help identify the origin of the parasites and subsequently inform public health responses. Genomic data on drug resistance markers and genotypes could support identification of imported cases, transmission foci and parasite migration, including in post-elimination settings in the region.

In an example of outbreak investigation from Peru, determining the parasite origin was only possible because of the rich data that existed – more than a decade of longitudinal data relevant to understanding the malaria parasites in the region. In the Peruvian Amazon, there was emergence of a drug resistance profile, Bv1 clonal lineage, that was distinctly different from the previous genotype found in the region. The Bv1 lineage profile posed a significant problem because the strain is multidrug-resistant and escapes detection by *Pfhrp2*-based RDTs secondary to *pfhrp2* and *pfhrp3* deletions. The hypothesis was that the Bv1 strain had emerged as a highly successful parasite lineage for transmission by different vectors and had contributed to the increased malaria burden recently observed in some Amazonian regions. Genetic connectivity was found between *P. falciparum* populations in Colombia and Ecuador, where there were also outbreaks. This is a critical use case scenario for understanding gene flow within a region, considering the significant impact resistance emergence could have on case detection and management.

The presentation highlighted an example of genetic epidemiology being used to identify imported cases to Guatemala in UN Peacekeepers who had spent nine months in the DRC. Genotyping showed that the infections in the returning peacekeepers were caused by parasites that were genetically related to DRC parasites and distinct from local Guatemalan parasites. This finding supported the case classification as imported (Fig. 12) and led to the implementation of a screening policy, treatment

protocols and prophylaxis in peacekeepers. Similarly, confirming the parasite origin of imported cases in non-endemic countries and understanding onward transmission can inform prevention guidelines for travellers. These analyses, among others, were successful due to regional partnerships, a longitudinal database, and the availability of data from multiple countries in the PAHO region.

Fig. 12. Neighbour-joining tree of three P. falciparum populations showing genetic connectivity between peacekeepers' parasites and DRC parasites *(12)*

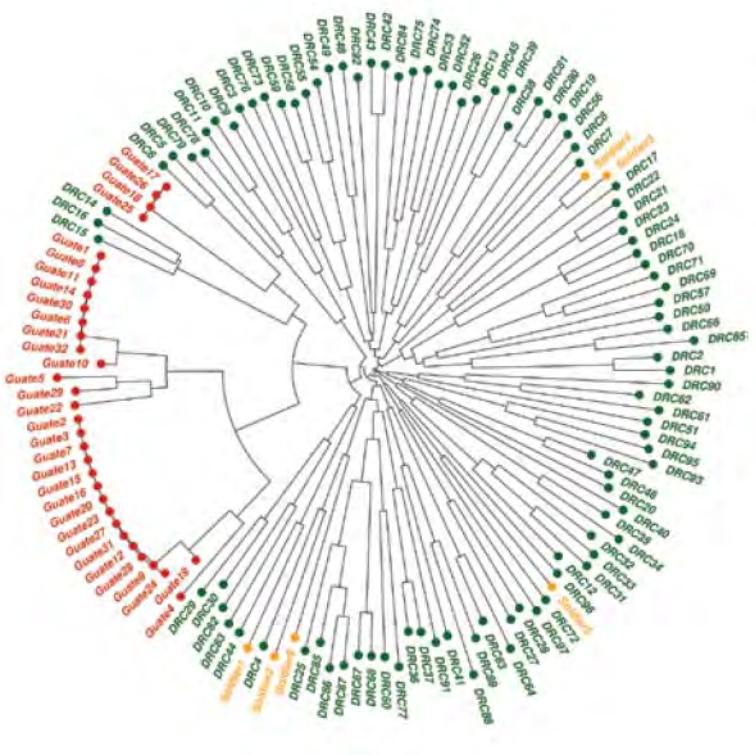


Figure 3. Neighbor-joining tree of 3 *Plasmodium falciparum* populations. Prefixes of genomes indicate parasite origins: Green text indicates parasite populations from the Democratic Republic of the Congo (DRC); orange indicates parasite populations detected in soldiers who were returning from the DRC to Guatemala; red indicates parasite populations from Guatemala.

3.6b. Use of genetic evidence in Greece

Presenter: Danai Pervanidou

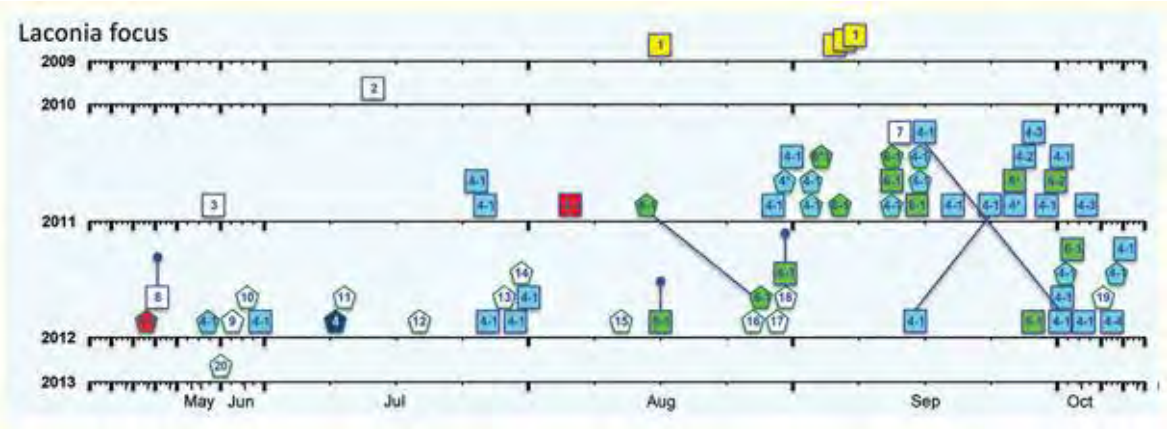
In Greece, there were several examples of genomic data supporting risk assessments, decision-making and case classification in the NMCP. Greece – a malaria-free country since 1974 – reports between 20 and 110 imported cases per year. A high receptivity risk combined with influxes of migrants from the Indian subcontinent has led to sporadic introductions and local acquisitions of *P. vivax* cases and one event of indigenous transmission in a particular area in 2011–2012. Given that this combination of continuous recording of imported cases and high receptivity risk increases the country's risk for malaria reintroduction, it became essential to establish an action plan for the management of malaria. The action plan is supervised by a multisectoral national committee and includes a series of prevention and response activities. In this context, the use of both genetic and epidemiologic data has supported risk assessment, surveillance and response.[4]

A key issue for Greece is that the WHO classification and definition of an introduced case requires documentation of the index imported case, which is often difficult to detect. Vulnerable populations, including refugees and undocumented migrants from malaria-endemic countries carrying out seasonal agricultural work, pose a local risk for malaria reintroduction (especially when the parasite is imported into a vulnerable area). However, due to barriers in vulnerable individuals accessing the

[4] For more information on the historical context and current epidemiological surveillance of malaria by the National Public Health Organization in Greece, see: https://eody.gov.gr/en/disease/malaria/.

health care system (due to e.g. fear of arrest/deportation, suspicion of the government and health services, language barriers, transport difficulties, etc.), their high turnover from one area to another, and mild manifestations of *P. vivax* relapses, it is often difficult to detect and record all imported cases that may have led to or will lead to introduced cases. As a result, the epidemiological criteria for classification of an imported case were adapted to account for these facts. The adapted criteria considered all *P. vivax* cases in migrants from endemic countries with symptom onset within three years post-arrival to be imported cases. It was necessary to apply genomic epidemiology to improve case classification and understanding of local malaria transmission routes. One case study in an area with a cluster of *P. vivax* malaria cases (2011–2012) among both migrants from endemic countries and Greek residents sought to distinguish between imported and locally acquired cases in order to understand transmission routes and risk in the community, and interrupt local transmission. Following genotyping, some of the cases were reclassified from imported to locally acquired because evidence suggested that there were clusters of linked cases based on similar haplotypes (Fig. 13). Similarly, in another case study that genotyped *P. vivax* cases occurring in two neighbouring households, genomic data allowed for reclassification of the cases (migrants from endemic countries) as locally acquired even though they were initially classified as imported; all cases, however, had the same haplotypes. While some of these analyses were conducted retrospectively, the approach could still be used to assess the transmission risk and inform timely decision-making and response within the programme.

Fig. 13. Clusters revealed through genotyping, Evrotas, 2011–2012



It is important to note that, in Greece, genotyping enabled a more comprehensive understanding of the malaria transmission risk in the country. Multiple importations and distinct introductions were confirmed, inferred from the detection of significant haplotype diversity and identification of small clusters. Insight into the transmission chains led to confirmation that there was no ongoing local transmission. Understanding the epidemiological situation facilitated decision-making that enhanced efforts to prevent the re-establishment of *P. vivax* in specific areas. Guided responses included mass drug administration programmes among migrants in high-risk areas (i.e., the area with the indigenous transmission event in 2011–2012, and the area with the cluster of introduced cases in two neighbouring households); reclassification of local risk levels to enhance the surveillance activities of the national malaria prevention programme; and intensification of response including proactive case finding and vector control in areas of high risk. In some cases, however, there were still limitations because the link between cases could not be confirmed and there was no supporting evidence to understand transmission routes. Questions that emerged included the possibility that the cases were linked by the same haplotype that was common in a particular area of the world, but were not linked epidemiologically; in other words, the cases were not in the same transmission chain. In this case, it was not possible to properly interpret the genetic analyses due to i) the need for more information on *P. vivax* genetics, including the geographical distribution and frequency of certain haplotypes in malaria-endemic countries, and ii) the lack of standardization of the loci used for genotyping, which is

required for cross-border comparisons and to provide a better interpretation of results. Moreover, knowledge is limited on the impact and potential role of the local vector population, and the species vectorial capacity of various imported *P. vivax* strains.

3.6c. Use of genetic evidence in China

Presenter: Junhu Chen

The National Institute of Parasitic Diseases, China CDC also presented significant use cases for genetic epidemiology in documenting progress towards elimination. One key focus was in understanding the parasite population in order to discern regional gene flow from introduced parasites and support case investigations. A use case that worked involved determining parasite relatedness in situations where cases were suspected to be linked by a local vector through nosocomial transmission, but genomic data were required for confirmation. Similarly, genomic data were able to provide evidence of transmission where conventional epidemiology could not in the investigation of a local case with no travel history in an area considered *P. falciparum*-free for two decades. In general, population and comparative analyses are effective when supporting genomic data can aid in case classification, determine parasite relatedness and trace geographic origin using IBD analyses. Limitations still exist depending on the parasite density, complexity of infection (COI) and sample size. In addition, access to a database of parasite genomes for comparison is key to identifying potential links, migration patterns and the geographic origin of parasites in supporting the case classification of imported and local transmission.

3.6d. Discussion

The PAHO region, Greece and China have different malaria transmission settings, but there are similarities in how genetic epidemiology has been applied to support NMCPs to identify transmission foci, determine risk of transmission and improve case classification. The discovery of *pfhrp2/3* deletions among clinical isolates in Peru in 2008 prompted retrospective investigations and prospective surveys in multiple neighbouring countries, including Brazil, Bolivia, Guyana, Honduras, Suriname and Colombia *(14)*. These findings directly informed policy around the use of RDTs in the PAHO region. While it is clear that the application of genetic epidemiology is useful in local settings, the need for a data repository of available local and global genomic data, as well as a network to facilitate data sharing is evident. Although genetic epidemiology allows for increased precision in case classification, there are concerns over the lack of standardization of methods for genotyping and subsequent data analysis across different geographical settings. It is also necessary to understand the local environment and susceptibility for transmission risk, so that this information can be used in conjunction with conventional and genetic epidemiology data to inform programmatic or policy decisions.

3.6e. Imported versus local transmission

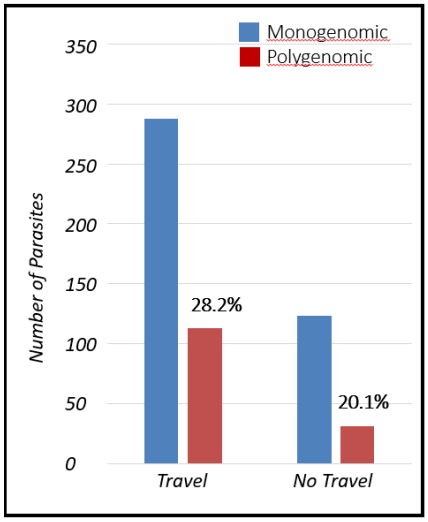
Presenter: Sarah Volkman

For NMCPs, the issues of importation and identification of transmission foci are important for tracking elimination progress and maintaining status as a malaria-free country. To further understand how genomic epidemiology can support case classification, it is necessary to first understand how parasite populations change with changing transmission intensity. As transmission intensity decreases from high to low, there is decreased COI, increased proportion of monogenomic infections (COI=1), appearance of clonal parasites and persistence of clonal lineages. This means that measures of parasite relatedness and connectivity can be used to understand transmission in an area and signify programme progress.

Genetic relatedness is measured using metrics of identity by state (IBS) and identical by descent (IBD): alleles that are genetically the same, and alleles that come from a common ancestor, respectively. Methods can be used to estimate IBD from IBS. This requires a number of informative markers

(molecular barcode genotyping) that vary depending on the level of transmission in the geographic area under examination. A barcode is considered informative for relatedness by IBS at >0.95 relatedness. When using this measure in a low transmission setting, relatedness can serve as a key indicator for distinguishing imported and local transmission and understanding the persistence of transmission in the area. For example, in Senegal, it was determined that there was an increased likelihood of polygenomic infections in travellers versus infections in households with no travel history, which were more likely to be genetically similar (Fig. 14). Additionally, there were identical parasites persisting across multiple years, which suggested maintenance of local transmission, albeit at low levels.

Fig. 14. Increased likelihood of polygenomic infections from travellers in a low transmission region: Richard Toll; Senegal



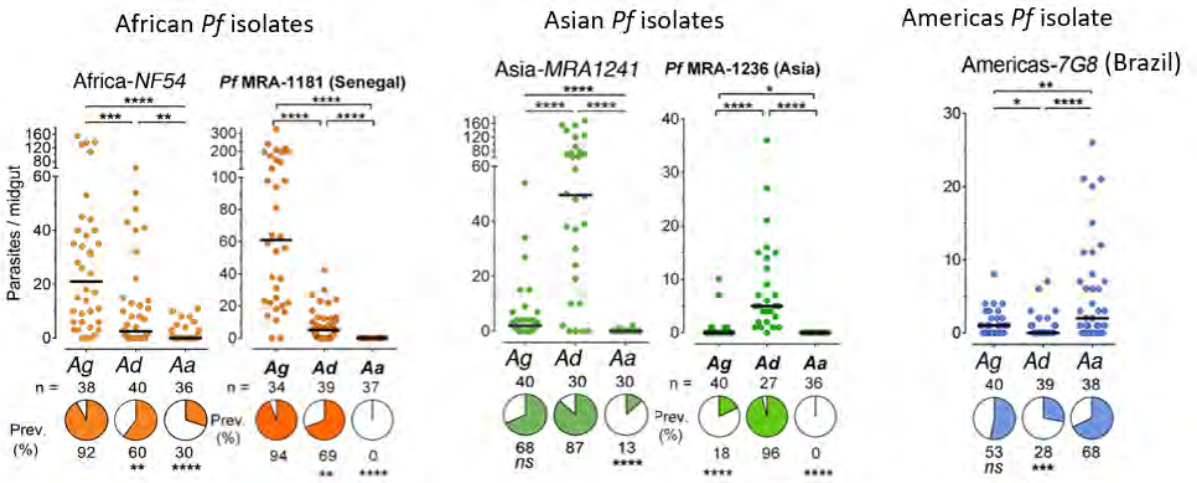
The ability to distinguish between imported and local infections can be critical to elimination and provides evidence for decreasing COI in parasite populations. Sequencing/amplicon data can reveal genetic connectivity to resolve questions in complex transmission settings; however, there is still a need for more genetic markers to expand the use of this methodology. In the case of Senegal, a 24 SNP barcode was used to characterize parasites. While these data were consistent with conventional epidemiologic data in terms of importation, directionality could not be confirmed. In this case, good traditional surveillance was necessary to ensure accurate interpretation of the genomic data.

3.6f. Pfs47 SNPs as a risk indicator for transmission of imported malaria

Presenter: Alvaro Molina-Cruz

An alternative approach to understanding receptivity risk for imported and onward indigenous transmission of malaria is to investigate parasite markers in parasite–vector interactions that determine whether the parasite can successfully infect the mosquito. A target of interest is *Pfs47*, which allows the ookinete to evade the immune response of the mosquito midgut and successfully develop into an oocyst. The allele is polymorphic with signatures of natural selection relevant to the geographic origin of the parasite. *P. falciparum* isolates are more compatible with *Anopheles* species from their region of origin (Fig. 15). This is related to parasite evolution as a result of both natural and forced human migration through which the parasite, but not the vector, was moved to different regions of the world. The allele is then linked to parasite development in the mosquito with adaptations in local mosquito populations within their respective regions globally. *Pfs47* SNPs can therefore be used to predict the transmission risk of imported *P. falciparum* and help establish its geographic origin. More data are needed to discern the boundaries for changes in which haplotypes are more or less prevalent. Additional research is needed to develop the evidence base for this phenomenon in *P. vivax.*

Fig. 15. *Pf* isolates are more compatible with *Anopheles* species from their region of origin *(13)*



3.6g. Discussion

The approach used for importation classification in low transmission settings in Senegal was based on the detection of decreased genetic diversity in local parasite populations. It is evident that more research is needed to build the evidence base on the underlying diversity of the local parasite population. In areas where knowledge of the local parasite population is poor, it will not be possible to identify imported cases, despite observations of decreasing polygenomic infections. In the case of *Pfs47*, this marker can offer information on the geographical origin of the parasite and whether the local mosquito populations are receptive to the genotype – a factor that could lead to ongoing transmission from imported cases.

3.7. Session 6: Data standardization, modelling and use

Facilitated by Abdisalan Noor

It is critical that genomics data be accessible so that important policy questions can be explored and platforms developed in order to eventually provide information products that are relevant to national malaria programmes. While the collection of these data is likely to remain within the realm of research in the near future, more routine processes for data assembly will increasingly become the main source of such data. This poses logistical as well as governance and ethical issues. Existing platforms for drug and insecticide resistance surveillance monitoring could also function as effective mechanisms for collecting genetic samples. As sample collection moves into the realm of routine surveillance systems, the burden on the health system and the ownership ethics involved in collecting samples in non-study settings will become important considerations. This will require the development of protocols and potentially normative guidance to advise countries on the way forward. Appropriate, statistical, geospatial and mathematical analysis methods should be explored so that results can be packaged in a way that is relevant to policy.

3.7a. Combining modelling and genomic surveillance data: insights for malaria elimination campaigns

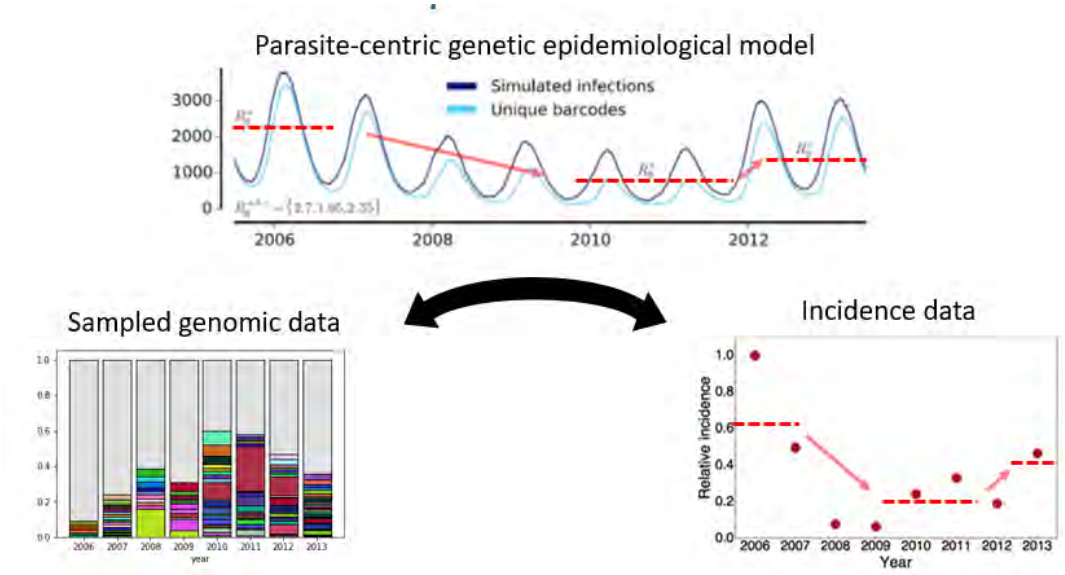
Presenter: Albert Lee

Integrating mathematical modelling can add value to genomic surveillance by bridging gaps when surveillance is limited, unifying information from multiple data sources via a common modelling framework, and pressure-testing the interpretation of genetic signals. Genetic models have been

shown to achieve actionable outputs, for example, linking R_0 and other epidemiological indicators to genomic signals and characterizing spatiotemporal patterns to estimate connectivity.

Transmission can be estimated using dynamic genetic models that build on connections with transmission indicators. For example, COI is an important genetic indicator, and its correlation with R_0 has been assessed. By simulating biological mechanisms, it is then possible to build on an understanding of parasite genetics to find order in complex genetic relationships and test theories against the data to determine where they may be most effective. This means that modelling may simulate trends in transmission without input from incidence data by using sampled genomic data from local parasite populations (Fig. 16). There are still challenges and limitations to this approach, such as the large number of components required to produce dynamic models that must be thoroughly tested, the dependency on priors that must be well understood and data-driven, and uncertainties in inputs that must be propagated to uncertainties in outputs. These issues can be resolved through close collaboration with local experts and a solid mathematical framework for uncertainty quantification.

Fig. 16. Parasite-centric genomic model reproduces trends in transmission without input from incidence data



Genetics improves the differentiation between importation and localized transmission. For example, models can provide a detailed view of local transmission properties by examining spatiotemporal correlations with links between strains from multiple infections. Positions of clonal infections over time enable estimates of dispersal velocity in emergent strains, and a mechanistic transmission model can then link dispersal velocity to spatial connectivity. The limitation, however, is that while models can estimate dispersal velocity to understand local versus imported transmission in relatively small geographic areas, this would require very robust data to ensure accuracy. The models are based on the parasites' clonal expansion but have yet to incorporate the effects of vector biology and human movement.

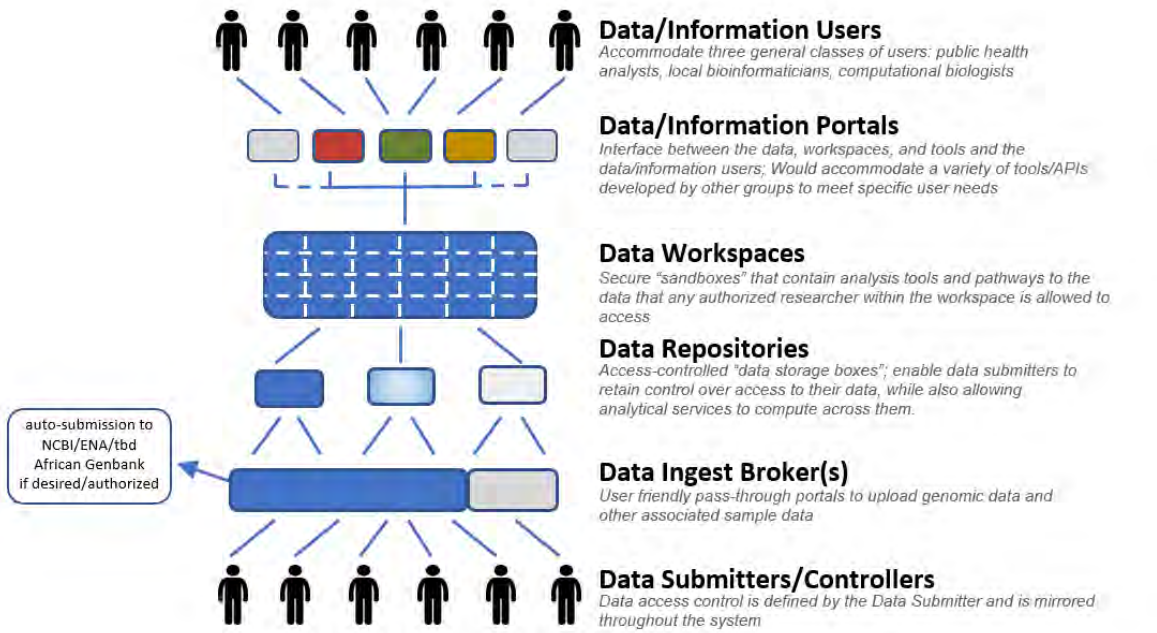
Genetic models can improve the precision and efficiency of surveillance programmes and the understanding of local parasite populations and patterns of transmission. With further development, such methods may allow for predictive modelling to identify hotspots and target interventions. The availability of comprehensive data will enable the training of such models to reduce the number of assumptions that need to be made and improve the accuracy of model projections. This will allow future surveillance efforts to be more effective with sparse data.

3.7b. Data standardization and translation for use in routine surveillance – a strategy for scale-up

Presenter: Bronwyn MacInnis

Across all potential use cases for malaria genetic epidemiology, one thing is exceedingly clear: the need for harmonization between data types (WGS, amplicon sequencing and genotyping) to allow for comparisons within and between countries, a common platform for data storage, analysis and reporting (Fig. 16), and agreements on data sharing.

Fig. 16. A Data System Concept for Genomic Pathogen Surveillance and Epidemic Preparedness



Considerations for the types of samples required, whether sequencing is necessary, and whether analysis requires specialized expertise all have implications for whether implementation and scale-up are possible at country level. Scaling up genomic data generation would require a coordinated multi-country effort with international partners and harmonization of core analytical workflows. A cloud-based, access-controlled data system for data storage has been suggested as a possibility to ensure country-level access control that could be deployed locally. This would remove the need for substantial computing infrastructure, downloading, tracking and version control. It is important for any system introduced to be adaptable to future needs as the field progresses.

The purpose of data sharing also needs to be considered – e.g., country-level public health programme needs versus academic research interests – along with the necessity to share data between countries, especially bordering countries. While many use case applications of genomic data require data sharing across country borders, many do not. Within-country applications, or those requiring bilateral data sharing, could be developed while open data sharing terms are considered and agreed. In approaches taken by other programmes, such as the polio laboratory network, it took a decade from proof of concept to establish a data sharing network. WHO has drafted a code of conduct for open and timely sharing of pathogen sequence data during outbreaks of infectious diseases. Simplified data sharing and communication already occur within the genomics community, which implies willingness to engage in informal sharing and the potential to introduce more refined options to solidify sharing mechanisms in a more robust fashion.

A recent Lassa fever outbreak in Nigeria was used as an example to demonstrate how rapid sequencing, analysis and data sharing helped answer the question of whether the outbreak was due to a new variant, due to a more virulent strain of the virus, or due to increased human to human transmission. Comparison of the viruses isolated from patients during the outbreak were compared to genomic sequences from viruses found elsewhere in Nigeria and in other countries. Results showed that there were multiple introductions of genetically independent viruses similar to known lineages in Nigeria, which excluded human to human transmission and the possibility of introduction of a new variant strain. The findings were shared with the Nigerian CDC and Lassa fever clinicians, and genomic data were released openly to the scientific community in real-time.

The key question is: How practical is it to scale up the use of malaria genomics in its current state? Some countries already have routine systems in place, but most high-burden countries do not have capacity to conduct sequencing, analyse data and use molecular epidemiology in the field. NMCPs have the opportunity to influence the approach for a way forward, but this requires consensus among partner organizations and countries to ensure that harmonization in the next steps occurs.

4. Working groups – Resistance, transmission, elimination and data collection: priority use cases for programme implementation

To further discuss potential use cases for malaria genetic epidemiology, two working groups were established: 1) Surveillance for *pfhrp 2/3* deletions and drug and insecticide resistance, and 2) Transmission dynamics across the transmission continuum and elimination. The objectives were to focus on research questions and use cases related to gene flow, including issues relevant to elimination settings, and to develop targets for the next 6–12 months, 1–2 years, 3–5 years and 5–10 years either for implementing the use cases as approaches to malaria control or for identifying areas that require more research. The groups also discussed where genomic surveillance would be most useful for policy, strategy and programme implementation; if there is evidence available for WHO review or a timeline for when information will become available; and what approaches would be best for data collection and interpretation in clinical, public health, surveillance and laboratory settings and what challenges are foreseen. During the group work, participants identified priorities for the application of genetic epidemiology in the detection and control of drug and insecticide resistance and transmission. These priorities are outlined below, while detailed information on operational use, field sampling and laboratory methods, ethics and data sharing, added value over conventional epidemiological methods, and challenges for implementation are provided in the supplementary tables (see Annex 1).

4.1. Surveillance for *pfhrp2/3* deletions and drug and insecticide resistance

In evaluating potential use cases for genomic surveillance of drug and insecticide resistance, there was a need to generate additional evidence and notable challenges for implementation. There were two use cases/applications for the surveillance of *pfhrp2/3* deletions or spread of drug resistance that were deemed ready for immediate action; the remaining use cases will require additional evidence for action in the medium term of 1–2 years or 3–5 years.

4.1.a. For immediate action (6–12 months)

Surveillance of *pfhrp2/3* deletions

- There is sufficient evidence from several countries to show that deletions of *pfhrp2 +/- pfhrp3* can cause false-negative HRP2-RDT results and that these parasites can become dominant in the parasite population. WHO has developed recommendations for investigating suspected false-negative RDTs due to *pfhrp2/3* deletions, as well as indications for conducting surveys, survey templates and criteria for when countries should switch to non-HRP2-exclusive RDTs. To support high-quality and rapid molecular analysis, WHO has also established a network of

reference laboratories experienced in *pfhrp2/3* genotyping and a proficiency testing scheme for malaria NAAT that includes *pfhrp2/3* deleted parasites. Until alternative diagnostic tests that can match the performance, stability and demand of HRP2-RDTs become available, surveillance for *pfhrp2/3* deletions across all epidemiological settings is essential for detecting areas where RDTs are failing and maintaining confidence in HRP2-RDT results.

- **Challenges for implementation:** Although not likely to be the only factor, the use of HRP2-RDTs themselves is expected to be driving the selection for *pfhrp2* deletions. The *pfhrp2*-negative parasites in Eritrea and Peru showed distinct haplotypes that strongly suggested de novo development of these parasites in both locations. Such development would imply that all malaria-endemic areas are at risk and that there is an urgent need to map the prevalence of *pfhrp2*-negative parasites to inform case management policy. The key challenge then becomes the mobilization of resources to conduct such mapping.

Monitoring changes in frequencies of molecular markers of drug resistance over time and space

- There is sufficient evidence to show that molecular markers can be used to monitor changes in drug resistance and *pfhrp2/3* deletions in parasite populations over space and time. This is essential for detecting populations at risk of treatment failure or under-detection by RDTs in order to subsequently inform first-line drug policy decisions (ensuring that effective treatment is given to patients) and ensure that patients can be adequately diagnosed. While passive surveillance is acceptable, active sampling biannually or annually using dried blood spots would be desirable in order to rapidly detect changes in drug resistance. Routine monitoring should be implemented at the appropriate administrative level, which is relevant for the implementation of national drug policies. This approach is less expensive and timelier than a therapeutic efficacy study (TES).

- **Challenges for implementation:** Countries require clear guidance, training and capacity-building on the establishment of an appropriate spatial sampling strategy, methods for amplicon sequencing or other genotyping methods, and data generation, analysis and interpretation – all of which could prove costly in the short term. Clear procedures also need to be developed to ensure that policies on first-line drugs can be modified rapidly in response to changing resistance patterns and implemented in the field in a timely manner.

4.1.b. For medium-term action (1–2 years)

Determining the origins of drug resistance

- Determining the origins of drug resistance can facilitate the monitoring of the spread of resistance within and between countries. By monitoring haplotypes associated with drug resistance mutations from samples on a routine basis and comparing them over time and across regions, it is possible to determine if drug resistance is emerging locally or spreading – something that is difficult to infer using standard epidemiological approaches. Identifying populations at risk can inform regional drug policies and ensure interventions are targeted to contain resistance.

- **Challenges for implementation**: More research is needed to identify molecular markers in different geographical settings, along with a database of parasite samples across multiple geographic regions that can be used for comparison in analyses. Outsourcing of WGS may be necessary to generate the data for establishing such a database, as well as data sharing agreements within and between countries. In addition to the challenges outlined for use case 4.1.a, further research is needed, followed by clear guidance on public health responses to emerging resistance versus spread.

Determining the number and spatial distribution of sentinel sites needed to assess insecticide resistance and monitor new interventions

- This is important to improve the timeliness of surveillance of insecticide resistance, allocate adequate resources and monitor the impact of new interventions.
- **Challenges for implementation**: In many countries, there is a lack of entomological capacity, as well as a lack of the geospatial and entomological expertise required to develop a spatial sampling strategy. This means that clear guidance and technical support is required to build entomological capacity in countries, which would subsequently support related activities in genotyping.

4.1.c. For medium-term action (3-5 years)

Detecting changes in parasite population structure or signatures of positive selection

- Detecting changes in parasite population structure to determine whether there is anthropogenic impact from interventions or other selective pressures can help to identify populations at risk for emergence of resistance. It can also lead to early detection of emergence of new resistance mechanisms through identification of new resistance markers. This requires continuous longitudinal spatial sampling of populations over time.
- **Challenges for implementation:** In addition to the challenges outlined for case 4.1a, parasite genomic data from the same region over time or from nearby regions are required for comparison, which may take several years to establish. The current analytical approaches, such as IBD, also have some limitations in terms of determining the origin of resistant alleles. More research is required to determine whether these approaches can be used at the operational level rather than as a research tool, which is how they are currently being used.

Monitoring local species composition and changes over time

- Improved understanding of local species composition and changes over time can i) inform selection of vector control tools by identifying key vectors responsible for transmission, and ii) aid in assessing residual transmission and its implications for the effectiveness of interventions. Cross-sectional sampling over time at sentinel sites can reveal the heterogeneity in the local vector and parasite populations and support the development of other use cases, such as improving the understanding of resistance patterns and transmission dynamics in a region.
- **Challenges for implementation**: There is a lack of reference databases which combine mosquito genomes and phenotypic data for many species, as well as a lack of validated spatial density data for decision-making. There is also a lack of capacity to carry out basic entomology in many countries which is required for sample collection and accurate species identification.

Insecticide resistance surveillance

- Monitoring insecticide resistance allows for the targeting of specific interventions (e.g., pyrethroid-PBO nets) and resistance mechanisms (e.g., mixed-function oxidase (MFO) resistance mechanisms) over time. Such monitoring also enables programmes to assess the value of different insecticide resistance management strategies (e.g., IRS rotation, new types of ITNs, attractive toxic sugar baits). Using genotyping to detect insecticide resistance is quicker to implement than phenotypic assays that require rearing of larvae and although wild type adults can be used, it is possible resistance could be underestimated due to unknown age of the mosquito. With this approach, shifts in allele frequencies may be easier to detect than shifts in phenotype over short time periods.

- **Challenges for implementation**: There are currently few molecular markers identified that are associated with MFO resistance and therefore extensive research is required in this area. The number of sentinel sites required to assess insecticide resistance is also unknown, and there is a lack of reference genomes for many species. In terms of using the information to inform strategies, it is still necessary to assess the value of each strategy in the context of the NMCP's national malaria control strategy. More research is required to assess the effectiveness of the combination of different control strategies targeting the mosquito and the parasite. Furthermore, the presence of resistance alleles does not allow us to measure resistance intensity which means control interventions could still be valid despite genes that confer resistance being detected. To begin to bridge the gaps that exist in the capacity for data generation and analysis, establishment of a network approach would facilitate further progress.

4.2. Transmission

Use cases for malaria genomics in the context of gene flow for transmission to elimination include both parasite and vector dynamics. Generally, the use of genomic data in this context is viewed as confirmatory, or as an augmentation to traditional epidemiologic data, providing more precise information where gaps or discrepancies remain. A key concern, however, is that more evidence is needed to validate genomic data with respect to traditional epidemiology. With the use of different methods at different levels of confidence for interpreting genomic data, quality assurance and control are needed to standardize approaches and establish the evidence base to support policy and programmatic decision-making at a higher level.

4.2.a. For immediate action (6–12 months) to medium-term action (1–2 years)

Vector species dynamics

- For understanding vector dynamics, use cases include understanding vectorial capacity and vector competence to inform surveillance and control measures surrounding imported cases. This use case is also of importance for imported case management in countries with low transmission or in malaria-free countries with high receptivity risk for sustained introduced transmission. Understanding the local vector competence for imported malaria species can help to define risk and inform response strategies for outbreak prevention.
- **Challenges for implementation:** A basic molecular biology laboratory with trained personnel, which is incorporated into existing or newly developed entomology facility networks, and a map of local vector species would be required and could take some time to establish.

4.2.b. For long-term action (5–10 years)

Changes in transmission

- Genomic data can help to shed light on other changes or fluctuations in population dynamics that are not always clear, e.g., due to natural phenomena. Understanding changing transmission and being able to distinguish between natural fluctuations in parasite populations and the impact of interventions are important for future strategic planning.
- **Challenges for implementation:** There are challenges with interpretation using current methods; for example, do changes detected reflect those of the broader population? Extensive research using well designed studies is required.

Transmission intensity

- Understanding the levels of transmission intensity and transmission patterns with accuracy can inform stratification and malaria control strategies, detect persistent local transmission and help to establish a baseline of variation for future parasite population-genetics studies.
- **Challenges for implementation:** See challenges under elimination use cases.

Gene drive

- With increasing research on gene drive as a control strategy, it is necessary to map implementation of research and assess impact on local mosquito and parasite populations. Determining the necessary spatial resolution of gene drive in the context of natural selective pressures in the field would improve the precision and future applications of this approach.

4.3. Elimination

The applications for the use of genomic data in elimination settings are either immediately actionable or actionable within the next 1 to 2 years.

4.3.a. For immediate action (6–12 months)

Elimination and low transmission settings: case classification of local, introduced or imported cases

- In low transmission settings, accurate case classification is crucial to certify a country as malaria-free (certification). The use of genomic data can add precision to case classification (indigenous vs imported), providing a country with evidence demonstrating zero indigenous cases of malaria.

4.3.b. For medium-term action (1–2 years)

Elimination and low transmission settings: risk factors for local transmission and outbreak investigations

- In low transmission settings, genomics can also help to identify active foci, provide information on the origin of imported cases, identify high-risk groups for infection and for sustaining transmission ("hotpops"), and assess their contribution to onward transmission. Accurate data on whether local transmission is occurring, and identification of associated risk factors enable high-risk groups to be targeted with screening/awareness campaigns.
- Genomic data can help to determine how geographical areas may be linked through regular travel/importations. In considering progress towards elimination, it is important to generate data that help to elucidate parasite boundaries in a region, regardless of administrative borders, so that determination of origin and control measures can be implemented in relation to the parasite boundary rather than administrative borders. Genomic data could serve as supplemental to conventional epidemiologic data in understanding the movement of people. Determining cross-border connectivity of parasites will allow for a coordinated response between bordering countries, across artificial or porous borders, and inform relevant decision-making in a regional context.
- In outbreak investigations, genomic data can be used in conjunction with conventional epidemiology to confirm linkages between locally transmitted cases. This information can be used to direct public health resources appropriately and prevent unnecessary investigations or interventions.
- **Challenges for implementation:** There is a need for a local and global repository of genetic parasite sequences that can be queried and ideally integrated into existing databases within the malaria community in order to aid in the identification of parasite origins. This will take some time to establish. Standardization is necessary across data, genotyping and analysis types to enable comparison, along with established mechanisms for quality assurance and

control. Capacity-building for *timely* genotyping, analysis, interpretation and use of data in countries is required. Guidance will be needed on translating genetic data into information that can easily be used by control and elimination programmes, particularly as part of outbreak investigations.

5. Next steps

Several next steps were identified:

1. The table of research priority areas (Table A1) identified during this meeting should be made available online and updated on an annual basis by WHO with help from research networks and individuals.
2. A database of researchers and institutions involved in policy-relevant malaria genetic epidemiology studies should be developed by WHO, and this database should be updated annually.
3. Use cases share several overlapping themes across the spectrum of transmission in terms of understanding gene flow in insecticide and drug resistance. Studies should maximize these linkages so that common data generation platforms and samples can be used, wherever possible.
4. In addition to research studies, there are opportunities to explore drug and insecticide resistance monitoring sites: collecting genetic samples during case detection and investigations in elimination settings, and, in burden reduction settings, passive case detection systems and household surveys could become the mainstay for genomic surveillance. A structured approach that will not add any unnecessary burden on the health system is needed.
5. Stakeholders should work with researchers to ensure that study protocols are designed to generate evidence in formats relevant to policy and programmes. For example, studies exploring the relevance of genomic surveillance metrics must include a comparison to metrics currently recommended by WHO and used by countries in terms of their relevance, reliability, accuracy, precision, cost and sustainability. WHO to work with network of research during study design stage.
6. Established global databases should be harnessed to develop information products relevant for policy and country operations. WHO to work with groups such as Sanger Institute and BROAD on appropriate information products once policy relevance is established.
7. Investment in regional and national capacities for genetic epidemiology should be sought. WHO to work with researchers and funders such as BMGF on pathways to increased national capacity.

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Annex 1

Table A1 Summary of priority research areas and questions

SPREAD OF DRUG RESISTANCE AND *PFHRP 2/3* DELETIONS							
Evidence/use cases: SpotMALARIA, Plasmodium Diversity Network Africa (PDNA) *(1–3)*, MalariaGen Network *(4)*, GenRe-Mekong (Genetic reconnaissance to support malaria elimination in the GMS) *(5–7)*, Malawi *(8)*, Bangladesh, Mali *(9)*, Cambodia *(10–12)*, Thailand *(13,14)*, Lao People's Democratic Republic *(15)*, Myanmar, Viet Nam, China *(16)*, French Guiana *(17), Peru (18), Eritrea (19)*							
Use case/ application	**Operational component**	**Field sampling (methods, data source, spatial scale, frequency)**	**Laboratory (methods, standardization, expected advances)**	**Ethics and data sharing**	**Added value**	**Challenges for implementation**	**Immediate, medium- or long-term action**
1) Monitoring changes in frequencies of molecular markers of drug resistance over time and space	First-line drug policy decisions Identify populations at risk of treatment failure	Passive case detection Active sampling desirable Desired frequency: annual or semi-annual Dried blood spots Spatial sampling strategy should be relevant to implementation of national drug policies (e.g., district, province, or country level).	Amplicon sequencing or other genotyping methods	Data ownership: country owns primary data Aggregate data shared with the malaria community	Less expensive than TES Early warning of clinical failure Ability to genotype from dried blood spots Allows more dense sampling in time and space and at epidemiological scales	Unbiased population sampling – including establishment of appropriate spatial sampling strategy Nagoya protocol Countries need technical support and capacity-building to generate, store and analyse the data. Procurement and access to reagents. May need to rely on regional reference laboratories Costs Timeliness for modifying policies and implementation in the field	Immediate Evidence ready for submission to WHO for review within six months to one year

2) Identifying and monitoring changes in frequencies of *pfhrp2/3* deletions	Directly informs RDT selection for national programmes	Prospective surveys of symptomatic patients presenting to health facilities: survey templates available Parallel testing using HRP2 and pf-LDH RDTs or microscopy and collection of dried blood spots Prioritize HRP2 negative/pf-LDH or microscopy positive for *pfhrp2/3* genotyping Target countries with reports of *pfhrp2/3* deletions and neighbouring countries If >5% *Pf* cases are missed due to *pfhrp2/3* deletions, replace RDTs in the country; if <5%, repeat survey in 1–2 years	PCR to confirm *Pf* infection; *pfhrp2* and *pfhrp3* and at least two other single copy genes Flanking genes, serology, whole genome sequencing, next-generation sequencing optional	Country owns primary data and should establish MTA with international reference laboratory Aggregate data shared with the malaria community through WHO Malaria Threat Maps	As post-market surveillance and complaint reporting are weak in endemic countries, and confidence in RDTs remains fragile in many places, surveillance across all settings where HRP2 RDTs are in use is necessary to allow for early warning of *pfhrp2/3* deletions causing false-negative RDTs.	Financial resources to implement baseline surveys and monitoring	Immediate
3) Determining origins of drug resistance (independent emergence vs spread)	To guide targeting of interventions for containment of resistance & inform regional drug policies	Passive case detection Active sampling desirable Desired frequency: annual or semi-annual Dried blood spots	Amplicon sequencing or other genotyping methods followed by whole genome sequencing	Country owns primary data Aggregate data shared with the malaria community	Ease of field implementation Shared haplotypes associated with drug resistance mutations provide evidence of origins that may be difficult to infer using standard epidemiological approaches. Continuous sampling possible	Access to whole genome sequencing – outsourcing may be necessary A large database of parasites from multiple geographic regions needs to be available for comparison. More research to identify molecular markers in different geographical settings	Medium-term Evidence for WHO review likely to be ready within the next 1–2 years

4) Detecting changes in parasite population structure or signatures of positive selection	Identifying populations at risk for emergence of resistance Early detection of emergence of new resistance mechanisms through identification of new resistance markers	Passive case detection Active sampling desirable Sampling of populations over time, ideally annually or semi-annually Dried blood spots Spatial sampling strategy should be relevant to implementation of national drug policies (e.g., district, province, or country level).	Whole genome sequencing or genome-wide genotyping	Country owns primary data Aggregate data shared with the malaria community	Detection of emerging new resistance mechanisms Ease of field implementation Early warning of populations at risk for emergence of resistance Continuous sampling possible. Can make use of historical samples	Access to whole genome sequencing – outsourcing may be necessary Parasite genomic data from the same region over time or nearby regions need to be available for comparison.	Medium-term Evidence for WHO review likely to be ready within the next 3–5 years
SPREAD OF INSECTICIDE RESISTANCE							
Evidence/use cases: *Anopheles gambiae* 1000 Genomes Project *(20)*							
Use case/application	**Operational component**	**Field sampling (methods, data source, spatial scale, frequency)**	**Laboratory (methods, standardization, expected advances)**	**Ethics and data sharing**	**Added value**	**Challenges for implementation**	**Immediate, medium or long-term action**

1) Determine the number and spatial distribution of sentinel sites needed to assess insecticide resistance and monitor new interventions	Improved surveillance of insecticide resistance and impact of interventions	Larval sampling, adult sampling (traps, human landing catches) or rearing larvae to adults.	Amplicon sequencing or other genotyping methods for known resistance markers; whole genome sequencing on a subset Morphological identification and where necessary, standard PCR assays for species complexes, must be carried out before any sequencing is done WHO susceptibility tube assays	Country owns primary data Aggregate data shared with the malaria community	More timely monitoring and adequate resources allocated Inform gene drive development and deployment	Lack of entomological capacity in-country. Entomological capacity should be established first before genotyping can be implemented. Need for spatial sampling strategy (i.e., need geospatial expertise along with entomological expertise)	Medium-term 1–2 years

2) Monitoring local species composition and changes over time	Incrimination of key vectors Informing selection of vector control tools Assessing residual transmission and its implications for intervention effectiveness	To be informed by surveillance work (see above) Cross-sectional sampling over time Spatial density Sampling to be conducted at sentinel sites Human bloodmeal index Sporozoite infection rate	Amplicon sequencing or other genotyping methods for known resistance markers; whole genome sequencing on a subset Morphological identification and where necessary, standard PCR assays for species complexes, must be carried out before any sequencing is done ELISA can be used for vector incrimination	Country owns primary data Aggregate data shared with the malaria community	Inform gene drive development and deployment Simple to implement when local entomological expertise is lacking, or species complexes have not been formally defined	Lack of reference genomes for many species Lack of validated spatial density data for decision-making	Medium term 3–5 years

3) Insecticide resistance surveillance	Targeting of specific interventions (pyrethroid-PBO nets) and resistance mechanisms (e.g., MFO resistance mechanisms) over time Assessing the value of different insecticide resistance management strategies (e.g., IRS rotation, new types of ITNs, attractive toxic sugar baits)	Sampling over time Spatial density dependent on spatial insecticide exposure Larval sampling, adult sampling (inside and outside buildings to detect behavioural resistance) or rearing larvae to adults.	Amplicon sequencing or other genotyping methods for known resistance markers; whole genome sequencing on a subset Morphological identification and where necessary, standard PCR assays for species complexes, must be carried out before any sequencing is done WHO susceptibility tube assays	Country owns primary data Aggregate data shared with the malaria community	Simpler to implement than phenotypic assays requiring rearing of larvae Shifts in allele frequencies may be easier to detect than shifts in phenotype over short time periods.	No known markers for MFO-mediated resistance Feasibility of outsourcing whole genome sequencing Lack of reference genomes for many species	Medium-term 3–5 years to long-term 5–10 years
TRANSMISSION							
Change in transmission							
Evidence/use cases: *P. falciparum* Community Project, MalariaGEN Network - Pf3k, SpotMALARIA							
Use case/application	**Operational component**	**Field sampling (methods, data source, spatial scale, frequency)**	**Laboratory (methods, standardization, expected advances)**	**Ethics and data sharing**	**Added value**	**Challenges for implementation**	**Immediate, medium- or long-term action**

1) Is transmission changing and how do we interpret natural fluctuation?	Can remove need for some active case detection to save costs Ongoing entomological surveillance is essential	Epidemiological framework to benchmark	Whole genome sequencing or amplicon sequencing; requires measure of relatedness	Country information is sufficient	Independent, orthogonal metric of transmission, helps to triangulate current metrics. At low transmission, current measures lose dynamic range/sensitivity. Genomic metrics may be more sensitive. Complementary data to help understand other changes/fluctuations that are not always clear, e.g., due to natural phenomena	No validated framework for calibrating epidemiologically relevant metrics or for deriving them. How to design this experiment? Challenges with interpretation; do changes detected reflect those of the broader population?	Long-term Evidence for WHO review likely to be ready within the next 5–10 years
2) Are interventions making an impact?	Signals whether interventions are impacting parasite populations in target regions Orthogonal data type to measure incidence May be an earlier or more sensitive indicator (tbd)	As above	Whole genome sequencing or amplicon sequencing	As above	As above	As above	
TRANSMISSION INTENSITY							
Evidence/use cases: Senegal, Panama, Malawi							
Use case/application	**Operational component**	**Field sampling (methods, data source, spatial scale, frequency)**	**Laboratory (methods, standardization, expected advances)**	**Ethics and data sharing**	**Added value**	**Challenges for implementation**	**Immediate, medium- or long-term action**

1) What are the levels of transmission intensity? Is it possible to develop a standalone genomic metric of transmission?	Inform stratification and malaria control strategies	A local and global repository of genetic sequences that can be queried and ideally integrated with existing databases within the malaria community to inform parasite origins	Whole genome sequencing and amplicon sequencing	Country information can be sufficient for identification of local transmission but requires shared data to identify source of imported infections.	Understand transmission intensity with more accuracy and better target malaria control	Standardization across data, genotyping and analysis types for comparison Quality assurance/quality control How to harmonize, "what tool/approach" should I use? Establishing a global repository for sharing of parasite genetic sequences Capacity for *timely* genotyping, analysis, interpretation and use of data in country Translation of genetic data into information that can easily be used for control and elimination programmes	Long-term
2) What is R_0 in different populations?	Predict spread and identify risk groups	As above	As above	As above		As above	
VECTOR SPECIES DYNAMICS							
Evidence/use cases: The MalariaGen Vector Observatory, Ag1000G Consortium							
Use case/application	**Operational component**	**Field sampling (methods, data source, spatial scale, frequency)**	**Laboratory (methods, standardization, expected advances)**	**Ethics and data sharing**	**Added value**	**Challenges for implementation**	**Immediate, medium- or long-term action**

1) What are the population dynamics of invasive vectors in Africa and other parts of the world? e.g. *An. Stephensi* in Djibouti and Sri Lanka	Guide vector control interventions If single introduction and low levels of genetic diversity, then these mosquitos could be eradicated	Entomological collections in Djibouti and Ethiopia Morphological species identification	Molecular taxonomic identification and genotyping (PCR of ITS2, microsatellite and mitochondrial markers)			Requires a basic molecular biology lab with trained personnel	Immediate
2) How does it compare with its parent population?	As above	As above	As above	As above	As above	As above	
3) Understanding local vectoral infectivity for imported parasites	As above	As above	As above	As above	As above	Need map of local vector species	Medium-term 1–2 years
GENE DRIVE							
Evidence/use cases:							
1) Map vector species to then track changes from gene drive							Long-term

ELIMINATION (AND SOURCE OF INFECTION MORE BROADLY)							
Evidence/use cases: Greece, China, Guatemala, Peru, Colombia, Ecuador, Panama, Kingdom of Eswatini, northern Senegal, northern Namibia, Bangladesh. Pfs47 as a potential candidate. Could Greece be a good retrospective benchmarking proof of concept?							
Use case/application	**Operational component**	**Field sampling (methods, data source, spatial scale, frequency)**	**Laboratory (methods, standardization, expected advances)**	**Ethics and data sharing**	**Added value**	**Challenges for implementation**	**Immediate, medium- or long-term action**
1) Are new cases locally transmitted, introduced or imported?	Improve classification of cases as indigenous or imported (over travel history) Identify transmission foci/sources Gives additional information about parasite origin for imported cases Can we calculate a rate of importation? Identify high-risk groups for infection and for sustaining transmission (hotpops) Certification of malaria-free/demonstration of zero indigenous cases of malaria	A local and global repository of genetic sequences that can be queried and ideally integrated with existing databases within the malaria community to inform parasite origins Travel history data Routine malaria surveillance data for elimination settings, including case investigation data	Whole genome sequencing and amplicon sequencing Dried blood spot samples	Country information can be sufficient for identification of local transmission but requires shared data to identify source of imported infections from other regions in the country or other countries. May require data sharing between administrative boundaries within the country. Should respect governance that protects patient confidentiality	More accurate data/ higher resolution for case classification and identification of foci Allows targeting of high-risk groups with screening/awareness	Standardization across data, genotyping and analysis types for comparison Quality assurance/quality control Establishing a global repository for sharing of parasite genetic sequences Capacity for *timely* genotyping, analysis, interpretation and use of data in country Translation of genetic data into information that can easily be used for control and elimination programmes	Immediate (in some contexts)

2) Is there ongoing local transmission?	Possible information about linkages between locally transmitted cases Stratification of interventions, i.e., to deploy vector control if local transmission is ongoing	As above	As above	As above	Determine whether transmission is occurring with higher accuracy Determine the source/source region of imported infections	Standardization across data, genotyping and analysis types for comparison Quality assurance/quality control Establishing a global repository for sharing of parasite genetic sequences Capacity for *timely* genotyping, analysis, interpretation and use of data in country Translation of genetic data into information that can easily be used for control and elimination programmes	Medium-term 1–2 years
3) Mapping transmission chains and better defining risk of onward infection	Assess contribution to onward transmission Determine how geographical areas may be linked through regular travel/importations	As above	As above	As above	Predict spread and carry out resource planning/targeted interventions Determine cross-border connectivity of parasites allowing a coordinated response between bordering countries	As above	
4) Outbreak/cluster investigation	Validation of epi linkages (or not) Determine the source of outbreak (local vs imported)	As above	As above	As above	Direct public health resources appropriately or prevent unnecessary investigations or interventions	As above	

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Technical Consultation on the role of parasite and anopheline genetics in malaria surveillance

Surveillance Unit

Laura Anderson
Abdisalan Noor
Dyann Wirth

Malaria Policy Advisory Committee, 2019

Global **Malaria** Programme

World Health Organization

Background

- Emerging evidence shows that genetic epidemiology can create new opportunities for malaria surveillance, prevention and control
  - Mosquito genotyping for improved mechanisms for speciation, better understanding of vectorial capacity and monitoring of spread of insecticide resistance
  - Parasite genotyping for understanding of transmission intensity and gene flow, including drug resistance, *Pfhrp2/3* deletions and facilitating quantification of malaria importation risk

- Most work to date has been carried out in research settings with few examples on how malaria genetic epidemiology can be used to improve operational decisions made by NMCPs

Three day Technical Consultation 5 to 7 June

Approved by MPAC in October 2018

Main objectives

- To understand the role of genetic epidemiology (specifically parasite and anopheline genetic signals and gene flow) in malaria surveillance and control
- To define priority research questions that are relevant to policy and operational activities of national programmes

Other objectives

- Review existing evidence across the use cases of genetic epidemiology in malaria surveillance
- Identify key research questions relevant to policy and operational activities of national programmes for each use case
- Discuss appropriate study protocols and issues related to ethics, data sharing and coordination mechanisms

Deliverables

- A meeting report summarizing the content of the presentations, discussions and outcomes of the meeting
- A list of key research questions relevant to policy and operational activities of national programmes for each use case
- A work plan to implement the key action points of the meeting

Participants

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Surveillance

Prevention, Diagnostic and Treatment

Entomology and Vector Control

Elimination

Drugs, Efficacy and Resistance

WHO other

Global TB Programme

Polio

Infectious Hazard Management

Meeting process

5 sessions

1. Experience from other diseases

    - Polio: Elimination setting
    - Ebola: Outbreak setting
    - TB: Ongoing transmission setting

2. Malaria parasite, anopheline gene flow, modelling

3. Parasite gene flow and spread of drug resistance

4. Parasite and mosquito genetics to understand transmission intensity

5. Parasite and anopheline gene flows to understand importation and identify foci of transmission

Meeting process

Group work

Group 1: Surveillance of *pfhrp* 2/3 deletions and drug resistance

Group 2: Transmission and elimination

- What are the use cases where genetic data will be most useful for national malaria programme strategy and operations?
- Do we have adequate information to make policy recommendations?
- How best do we collect the required data (SoPs, coordination, timelines, data sharing, analysis)

Meeting process

Use case/application	Operational component	Field sampling (methods, data source, spatial scale, frequency)	Laboratory (methods, standardization, expected advances)	Ethics and data sharing	Added value	Challenges for implementation	Immediate, medium- or long-term action
1) Monitoring changes in frequencies of molecular markers of drug resistance over time and space	First-line drug policy decisions Identify populations at risk of treatment failure	Passive case detection Active sampling desirable Desired frequency: annual or semi-annual Dried blood spots Spatial sampling strategy should be relevant to implementation of national drug policies (e.g., district,	Amplicon sequencing or other genotyping methods	Data ownership: country owns primary data Aggregate data shared with the malaria community	Less expensive than TES Early warning of clinical failure Ability to genotype from dried blood spots Allows more dense sampling in time and space and at epidemiological scales	Unbiased population sampling – including establishment of appropriate spatial sampling strategy Nagoya protocol Countries need technical support and capacity-building to generate, store and analyse the data. Procurement and access to reagents. May need to rely on regional reference laboratories	Immediate Evidence ready for submission to WHO for review within six months to one year

Immediate

Evidence ready for submission to WHO for review within six months to one year

Medium-term

Evidence for WHO review likely to be ready within the next 1–2 years

Medium-term

Evidence for WHO review likely to be ready within the next 3–5 years

Long-term

Evidence for WHO review likely to be ready within the next 5–10 years

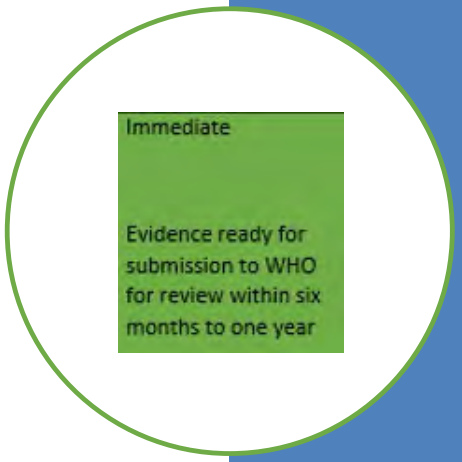
What we expect from MPAC

- Review and improve priority questions and next steps.
- Discuss and agree on the lead and coordinating roles of WHO in each of the next steps.
- Advise on proposals to use existing sentinel sites and passive case detection systems for sampling.

Priority Questions- *pfhrp2/3* deletions

Surveillance for *pfhrp2/3* deletions

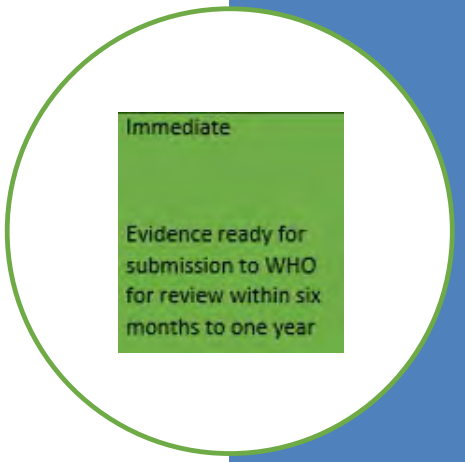
- Sufficient evidence from several countries to show that deletions of *pfhrp2 +/- pfhrp3* can cause false-negative HRP2-RDTs.
- WHO has developed recommendations on investigating suspected false negative RDTs due to *pfhrp2/3* deletions as well as indications for when countries should switch to non-HRP2-exclusive RDTs.
- WHO has established a network of reference laboratories experienced in *pfhrp2/3* genotyping and a proficiency testing scheme for malaria NAAT that includes pfhrp2/3 deleted parasites.
- Surveillance for *pfhrp2/3* deletions across all epidemiological settings is essential for maintaining confidence in HRP2-RDT results and detecting areas where RDTs are failing.



Priority Questions-Parasite drug resistance

Monitoring changes in frequencies of molecular markers of drug resistance over time and space

- Sufficient evidence to show that molecular markers can be used to monitor changes in drug resistance in parasite populations over space and time.
- Essential for detecting populations at risk of treatment failure in order to subsequently inform first-line drug policy decisions (ensuring that effective treatment is given to patients).
- **Routine monitoring** should be implemented at the appropriate administrative level, which is relevant for the implementation of national drug policies.



Priority Questions- Parasite drug resistance

Determining the origins of drug resistance

Medium-term

Evidence for WHO review likely to be ready within the next 1–2 years

- Determining the origins of drug resistance can facilitate the monitoring of the spread of resistance within and between countries.
- By monitoring haplotypes associated with drug resistance mutations from samples on a routine basis and comparing them over time and across regions, it is possible to determine if drug resistance is **emerging locally or spreading**
- Identifying populations at risk can inform regional drug policies and ensure interventions are targeted to contain resistance.

Detecting changes in parasite population structure or signatures of positive selection

Medium-term

Evidence for WHO review likely to be ready within the next 3–5 years

- Detecting changes in parasite population structure to determine whether there is **anthropogenic impact from interventions** or other **selective pressures** can help to identify populations at risk for emergence of resistance.
- Early detection of emergence of **new resistance mechanisms** through identification of **new resistance markers**.

Priority Questions- Insecticide resistance

Monitoring local species composition and changes over time

Medium-term

Evidence for WHO review likely to be ready within the next 3–5 years

- Improved understanding of local species composition and changes over time (gene flow within countries and between countries) can i) **inform selection of vector control tools** by identifying key **vectors responsible for transmission**, and ii) aid in assessing residual transmission and its implications for the effectiveness of interventions.

Insecticide resistance surveillance

Medium-term

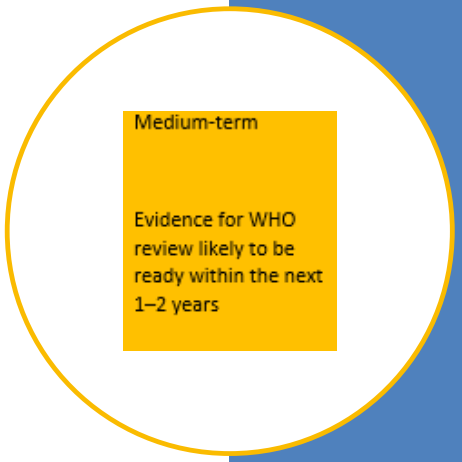
Evidence for WHO review likely to be ready within the next 3–5 years

- Monitoring insecticide resistance allows for the **targeting of specific interventions** (e.g., pyrethroid-PBO nets) and resistance mechanisms (e.g., mixed-function oxidase (MFO) resistance mechanisms) over time. Such monitoring also enables programmes to **assess the value** of different insecticide resistance management strategies (e.g., IRS rotation, new types of ITNs, attractive toxic sugar baits).

Priority Questions-Transmission

Vector species dynamics

- Understanding **vectorial capacity** and **vector competence** to inform surveillance and control measures surrounding imported cases.
- Imported case management in countries with **low transmission** or in **malaria-free** countries with **high receptivity ris**k for sustained introduced transmission.
- Understanding the **local vector competence** for **imported malaria species** can help to define risk and inform response strategies for outbreak prevention.



Priority Questions-Transmission

Changes in transmission

- Understanding changing transmission and being able to distinguish between **natural fluctuations in parasite populations** and the impact of interventions are important for future strategic planning.

Transmission intensity

- Understanding the levels of transmission intensity and transmission patterns with accuracy can **inform stratification and malaria control** strategies, detect **persistent local transmission** and help to establish a baseline of variation for future parasite population-genetics studies.

Gene drive

- With increasing research on gene drive as a control strategy, it is necessary to map implementation of research and assess impact on local mosquito and parasite populations.

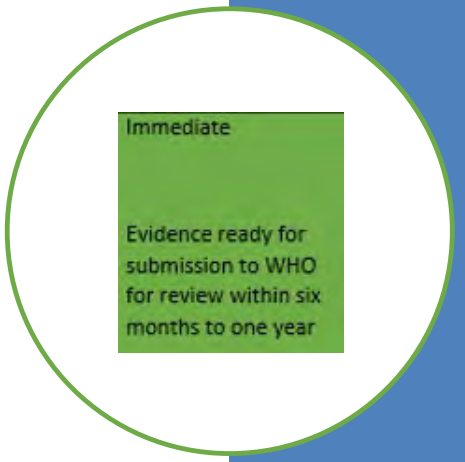
Long-term

Evidence for WHO review likely to be ready within the next 5–10 years

Priority Questions-Elimination

Elimination and low transmission settings: case classification of local, introduced or imported cases

- In low transmission settings, accurate case classification is crucial to certify a country as malaria-free (**certification**).
- The use of genomic data can add precision to **case classification** (indigenous vs imported), providing a country with evidence demonstrating zero indigenous cases of malaria.



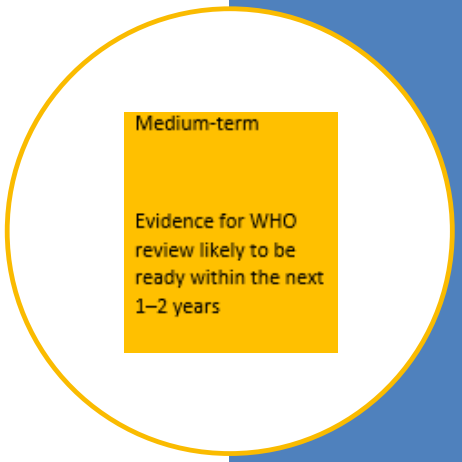
Priority Questions-Elimination

Elimination and low transmission settings: risk factors for local transmission and outbreak investigations

- In low transmission settings, genomics can also help to identify **active foci**, provide information on the **origin of imported cases**, identify **high-risk groups** for infection and for sustaining transmission ("hotpops"), and assess their contribution to onward transmission.

- Genomic data can help to determine how **geographical areas** may be **linked** through regular travel/importations. In considering progress towards elimination, it is important to generate data that help to elucidate **parasite boundaries** in a region, regardless of administrative borders, so that determination of origin and control measures can be implemented in relation to the parasite boundary rather than administrative borders.

- In **outbreak investigations**, genomic data can be used in conjunction with conventional epidemiology **to confirm linkages** between locally transmitted cases. This information can be used to **direct public health resources** appropriately and prevent unnecessary investigations or interventions.



Opportunities for which WHO should take a lead role

- WHO should develop and host a database of researchers and institutions involved in policy-relevant malaria genetic epidemiology studies, and this database should be updated annually.
- WHO should make the table of research priority areas identified during this meeting available online and update it on an annual basis with help from research networks and individuals.
- Evidence review groups should be convened in a timely manner as new evidence emerges.
- In addition to research studies, there are opportunities to explore drug and insecticide resistance monitoring sites, collecting genetic samples during case detection and investigations in elimination settings, and in burden reduction settings, passive case detection systems as well household surveys could become the mainstay for genomic surveillance. A structured approach that will not add unnecessary burden on health system is needed.
- Established global databases should be harnessed to **develop information product**s relevant for policy and country operations.

Opportunities for which WHO should take a coordination role

- Investment in regional and national capacities for genetic epidemiology should be sought.
- WHO should work with researchers to ensure that study protocols are designed to generate evidence in formats relevant to policy and programmes. For example, studies exploring the relevance of genomic surveillance metrics must include a comparison to metrics currently recommended by WHO and used by countries in terms of their relevance, reliability, accuracy, precision, cost and sustainability.
- Use cases share several overlapping themes across the spectrum of transmission in terms of understanding gene flow in insecticide and drug resistance. Studies should maximize these linkages so that common data generation platforms and samples can be used, wherever possible.

Challenges

- Countries need technical support and capacity-building to generate, store and analyse the data.
- Lack of reference genomes; a global database of comparable sequences is required which should be accessible for submitting data, querying (useful outputs for public health) and analysis (research).
- Nagoya protocol- Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization.
- Robust regional reference laboratory networks and outsourcing of genotyping/bioinformatics.
- Standardization across data, genotyping and analysis types for comparison.
- Quality assurance and control.
- Translation of genetic data into information that can easily be used for control and elimination programmes.

Thank you

Malaria Policy Advisory Committee Meeting
2–4 October 2019, Geneva, Switzerland
Background document for Session 7

World Health Organization

Meeting report of the WHO technical consultation on the spread of *Anopheles stephensi*

25–27 June 2019, Geneva, Switzerland

1. Background

In recent years, *Anopheles stephensi*, an efficient urban malaria vector for both *Plasmodium falciparum* and *P. vivax*, has been reported in four countries outside of the previously known geographical range, which was considered to be confined to certain countries in South-East Asia and large parts of the Arabian Peninsula.

The first detection outside the traditional geographical range was reported in Djibouti in 2012, in an area between Djibouti City and the Somalian border *(1).* A follow-up study from 2013 to 2017 confirmed the presence of *An. stephensi* mosquitoes in Djibouti City 2017 *(2).* In addition, in 2016, the vector was detected for the first time in Mannar Island in Sri Lanka, five years after the country achieved zero malaria transmission *(3).* Subsequently, *An. stephensi* was reported in Ethiopia's Somali Regional State, which borders Djibouti and Somalia *(4)* and, most recently, in two states in East Sudan (Ayman Ahmed, personal communication).

Experiences gained within the traditional geographical range of *An. stephensi* have shown that it can be a highly efficient malaria vector, particularly when it establishes itself in urban environments. Some of the *An. stephensi* specimens collected in Djibouti City were positive for *P. falciparum* sporozoites. The presence of this vector has been linked to the resurgence of malaria in Djibouti City.

The detection of *An. stephensi* in countries outside its established range poses a potential threat to malaria control and elimination. In Sri Lanka, the emergence of this vector could jeopardize efforts to prevent the reintroduction of malaria. In Africa, given the rapid and uncontrolled growth of cities, the potential establishment of this vector in urban environments could put at risk the reductions in malaria burden achieved since 2000.

2. Objectives of the technical consultation

The World Health Organization (WHO) plays a key role in monitoring threats to malaria control, elimination and prevention of re-establishment, and in providing guidance to Member States on how to manage these threats. Recognizing the emergence and spread of *An. stephensi* through the Horn of Africa and Sudan as a potential threat, WHO convened a Technical Consultation to assess the current evidence on this potential threat and to define a response strategy.

This document was prepared as a pre-read for the meeting of the Malaria Policy Advisory Committee and is not an official document of the World Health Organization.
WHO/CDS/GMP/MPAC/2019.14

3. Specific activities of the technical consultation

- To review published and unpublished evidence on the presence of *An. stephensi* outside of its traditional geographic range;
- To review efforts to model potential areas at risk for *An. stephensi* introduction and assess – to the extent feasible – the potential risk for further spread beyond the vector's previously reported geographic range;
- To review knowledge on *An. stephensi's* bionomics and biology, and analyse differences between vector populations in Asia and Africa to identify suitable control practices for each area where the vector is present;
- To review countries' experiences of controlling *An. stephensi* where it has been traditionally present, with the aim of identifying best practices and the main challenges in the control of this species;
- To review the status of *An. stephensi* resistance to different insecticide classes;
- To recommend surveillance and control strategies to address the threat posed by the spread of *An. stephensi* and surveillance indicators to assess the impact of control interventions.

4. Proceedings

This technical consultation was convened by WHO's Global Malaria Programme (GMP) on 25–27 June 2019. The agenda is provided in Annex 1 and the list of meeting participants in Annex 2.

4.1 Opening and orientation

The meeting was opened by Dr Jan Kolaczinski (WHO/GMP). Subsequently, all attendees introduced themselves in a session led by the chair, Dr Kezia Malm (National Malaria Control Programme, Ghana). The Declarations of Interest were disclosed to the advisors and committee members. Based on WHO's review of the declared interests, it was decided that none of the declarations constituted a conflict of interest in this context and that the considered experts could participate in the meeting, subject to the public disclosure of their interests. The Statement of Declarations of Interests was read out to the meeting participants and is provided in Annex 3.

As specified in the Terms of Reference, the meeting focused on the urgent need to review the current evidence on the distribution, surveillance and control of *An. stephensi*, followed by detailed discussions on the morphology, vector bionomics, potential areas at risk through modelling, and resistance to insecticides. Emphasis was placed on the need to successfully detect and control this invasive vector to prevent it from spreading to new geographic locations.

The sub-sections follow the specific meeting objectives and provide summaries of the various presentations and discussions over the course of the three-day meeting.

4.2 Summary of oral presentations

4.2.1 Current understanding on the spread of An. stephensi to new geographical areas

The oral presentations on day one commenced with Dr Manonath Marasinghe (Anti-Malaria Campaign, Colombo, Sri Lanka), who discussed the detection and spread of *An. stephensi* in Sri Lanka. Surveillance in Sri Lanka is focused on both coastal and inland rural and peri-urban environments. *An. stephensi* was first detected on Mannar Island in December 2016, leading to enhanced surveillance.

This presentation was followed by Dr Sinnathamby N. Surendran (University of Jaffna, Sri Lanka), who presented on the genotype and biotype of *An. stephensi* detected in Sri Lanka. The evidence shows that the type form is highly competent and capable of spreading malaria in rural and urban environments. The presentations noted the geographical locations where *An. stephensi* has been detected.

The next presentation was delivered by Professor Dr Michael Faulde (University Clinics, Bonn, Germany) on the introduction of *An. stephensi* to Djibouti and the ensuing resurgence of malaria. The presentation focused on the detection timeline of *An. stephensi* in Djibouti and other countries in the Horn of Africa, and potential urban hotspots.

The next set of presentations focused on Ethiopia. Dr Solomon Yared (Jigjiga University, Ethiopia) and Dr Tamar Carter (Baylor University, United States of America) presented their findings on the detection and insecticide susceptibility of *An. stephensi* in eastern Ethiopia. The data showed the presence of many anopheline species in urban water reservoirs. Discussion then centred on the quality of field collection and identification. Dr Carter presented on the molecular analysis, showing that *An. stephensi* has high genetic diversity in Ethiopia and possibly has metabolic resistance to certain insecticides. Further discussion centred on future directions of population and molecular data.

Mr Ayman Ahmed (University of Khartoum, Sudan) delivered a presentation via phone, as he was unable to participate in person. He presented the first-ever report of *An. stephensi* in Sudan, where the vector was coincidentally identified as part of a study on insecticide resistance in malaria vectors. Morphological and sequence-based identification confirmed the presence of *An. stephensi* in two states in East Sudan.

The presentations continued with Dr Courtney Murdock (University of Georgia, USA), who presented her assessment of the environmental suitability of *An. stephensi* in African cities. Information focused on predictive population growth rates at optimal thermal conditions. This was followed by a presentation by Dr Marianne Sinka (University of Oxford, United Kingdom) on predicting the potential for *An. stephensi* invasion across sub-Saharan Africa. While explaining the predictive modelling, the presentation highlighted the knowledge gaps we currently face in understanding the geographical range of *An. stephensi*.

4.2.2 An. stephensi surveillance, control and invasion response

Dr Rajpal Yadav (Department of Control of Neglected Tropical Diseases, WHO, Geneva, Switzerland) presented on *An. stephensi* distribution and control in urban and rural areas of India, focusing on the prevalence and most effective surveillance techniques. This presentation was complemented by Dr Naveen Rai Tuli (Municipal Corporation of Delhi, India), who reported on the Urban Malaria Scheme in India, which is a unique surveillance and control programme for *An. stephensi* (and other mosquito species) in the city of Delhi. The presentation outlined the core activities of the programme, namely to increase community literacy and trust, and to carry out larval collection and methodical surveillance. This programme has been built to adapt to the ever-changing ecology of the mosquito population.

Dr Manonath Marasinghe (Anti-Malaria Campaign, Colombo, Sri Lanka) focused on the surveillance and control response in Sri Lanka. As in India, Sri Lanka implements enhanced surveillance activities that continuously check for presence of malaria vectors, including *An. stephensi.*

Colonel Dr Abdoulilah Ahmed Abdi (Counsellor to the President of Djibouti) presented on the surveillance and control practices in Djibouti. The work to date has focused on understanding the intersections of urbanization and human population movement with *An. stephensi* distribution.

Dr Ahmadali Enayati (Mazandaran University of Medical Sciences, Sari, Islamic Republic of Iran) presented on surveillance of insecticide resistance and the resulting control practices in Iran, where

similar surveillance and control practices are implemented as in other countries. Additionally, the presentation provided details on insecticide resistance of *An. stephensi* in Iran.

The next set of presentations focused on Ethiopia. Dr Meshesha Managido (PMI Vector Link Project, Ethiopia) presented on larval and adult surveys in 10 sites in eastern Ethiopia, illustrating how surveillance is being conducted. Fitsum Tadesse (Armauer Hansen Research Institute, Ministry of Health, Ethiopia) presented on the ability of *An. stephensi* collected in Ethiopia to transmit malaria parasites. Dr Delensaw Yewhalaw (Jimma University, Ethiopia) presented on plans for *An. stephensi* research in Jimma. Mr Mebrahtom Haile Zeweli (In Charge of Malaria Focal Point, Ethiopia) presented on the nation's response to this new vector, including information on the capacity of *An. stephensi* to be a malaria vector and the need to create a working group to coordinate the response against this invasive vector.

The next group of presentations dealt with the new tools for surveillance and control. Dr Mike Reddy (Microsoft Research) presented on Project 'Premonition', which is a scalable biome monitoring device. The device attempts to utilize artificial intelligence and novel sensor packages to detect, identify and capture mosquitoes and determine their genomic contents using advanced metagenomic methods to aid in the surveillance and control of a wide range of insects, one of which is *An. stephensi*. Dr Laura Norris (Bill & Melinda Gates Foundation) discussed the Foundation's research objectives in terms of investing in *An. stephensi* control. This led to a presentation by Dr Kevin Gorman (Oxitec Limited, United Kingdom) on Oxitec's genetic self-limiting gene-drive for *An. stephensi.* The self-limiting construct for *An. stephensi* that is currently under development is building on Oxitec's Friendly™ safe, non-biting male mosquitoes, which are designed to suppress local wild populations of disease-spreading mosquitoes. Friendly™ mosquitoes carry a self-limiting gene, which means that when Friendly™ mosquito males mate with wild females, their offspring inherit a copy of this gene, which prevents females from surviving to adulthood. Since these females do not mature to reproduce, there is a reduction in the wild pest population. Male offspring survive, carrying a copy of the self-limiting gene; in turn, these males pass the self-limiting gene to half of their offspring, of which female carriers of the gene cannot survive. Presence of the self-limiting gene thereby declines over time, potentially offering multiple but still self-limiting generations of suppression for every Friendly™ male mosquito released. The self-limiting construct embeds into the desired mosquito species lineage.

Urban malaria in Africa was presented by Professor Maureen Coetzee (University of Witwatersrand, Johannesburg, South Africa), who summarized and elucidated the relevant anopheline taxonomic studies on the continent of Africa.

4.3 Summary of presentations and discussions

4.3.1 Current known distribution

An. stephensi, a highly competent vector of *P. falciparum* and *P. vivax,* is considered an efficient vector of urban malaria. In parts of India, *An. stephensi* ('type' and 'intermediate' biological forms) has also emerged as an efficient vector in rural areas as a result of changing agricultural and water storage practices. The 'mysorensis' form is considered to be a poor vector, although it has been involved in malaria transmission in certain rural areas in Iran and Afghanistan. Until 2011, the reported distribution of the vector was confined to certain countries in South-East Asia and the Arabian Peninsula. Since then, the vector has been reported in Djibouti (2012–2017), Ethiopia (2016), Sri Lanka (2017) and most recently Sudan (2019, unpublished report). In the Horn of Africa, the vector seems to be spreading from its first site of detection in Djibouti to neighbouring countries.

4.3.2 The potential risk for further spread of An. stephensi

The outcomes of two model-based mapping exercises were presented, indicating that *An. stephensi* could potentially emerge and thrive in other urban environments throughout Africa. However, more

information is needed to reliably inform such modelling exercises to identify potential areas at risk and target surveillance and intervention responses accordingly. To aid in the development of mathematical and statistical models, a general consensus is needed on what data (variables) need to be collected and how these will be incorporated. Temperature evaluations and human population densities seem to be two critical variables. In addition, information on breeding habitat availability and actual mosquito densities will be needed to validate the models.

4.3.3 Bionomics and biology of An. stephensi

It is worth noting that *An. stephensi* somewhat resembles *An. gambiae s.l*. morphologically. The current identification key *(5)* used throughout Africa does not include *An. stephensi*, which raises concerns about underestimating its current geographic range. Professor Maureen Coetzee has updated the identification key, which will be published soon. The WHO Vector Alert on *An. stephensi,* published shortly after the Technical Consultation, contains a brief key to separate this vector from the *An. gambiae* complex. Work is also ongoing through the WHO Department of the Control of Neglected Tropical Diseases (NTD) in line with the International Health Regulations (IHR 2005) to ensure vector surveillance and control at points of entry. The IHR (2005) represent an agreement among 196 countries, including all WHO Member States, to work together for global health security. The ongoing work of NTD and IHR (2005) is targeted at assisting port officers and focuses on all vectors of human pathogens, including mosquitoes, mites, ticks, rodents and fleas. The work is supported by the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China with respect to the development of a pictorial vector identification platform that uses only basic keys. This platform, under development, is a web-based, "Points of Entry (PoE) Vector Identification" platform, housed in the WHO PAGNet-Ports, Airports and Ground Crossings Network (https://extranet.who.int/pagnet/). It is aimed to provide support to staff at PoE assigned for vector surveillance and control to identify main exotic and endemic vectors of human diseases via an online search tool and expert assistance. At the current stage, images of mosquito species have been uploaded to form a basic mosquito taxonomy (dichotomous keys) for identification of invasive mosquito species. A workshop has been implemented in a selected EMR country to introduce and pilot test the online Vector Identification platform to improve upon training before it is rolled out to other countries in the WHO regions. This global platform is envisaged to be shared with all state parties, which will assist national health authorities in decision making/early warning, as well as to initiate adequate public health control measures to counteract impact of vector borne diseases.

An. stephensi typically breeds in man-made containers or cisterns with clean water and appears to adapt itself quickly to the local environment, including adaptation to (i) cryptic habitats, including deep wells in extremely high temperatures during the dry season when malaria transmission normally reaches a seasonal low, (ii) polluted water, and (iii) saline water. Its immatures (i.e. larvae and pupae) often coexist in the same breeding sites as the immatures of *Aedes aegypti* and can coexist with immatures of *An. varuna* (Sri Lanka) and *An. arabiensis* (Ethiopia). It is worth noting that *An. stephensi* mosquitoes collected in Ethiopia were able to harbour *P. vivax* malaria in standard membrane-feeding assays, albeit in lower numbers than *An. arabiensis* (unpublished report).

4.3.4 Experiences of controlling An. stephensi

Experiences of controlling *An. stephensi* in Africa have been limited or absent. In India, Iran and Pakistan (the areas of the vector's original distribution), vector control has been achieved through:

- management of vector breeding sites in urban and peri-urban environments by removing breeding sites, sealing lids to water storage containers and treating water with WHO-prequalified chemical and/or biological larvicides;
- introduction of mechanical barriers (e.g., window and door screening) to prevent female mosquitoes from entering human dwellings;

- enactment/introduction of by-laws to regulate water storage practices and construction work so as to avoid creation of potential breeding sites;
- enforcement of IHR (2005) to ensure that airports and other points of entry/exit are free of vectors, specifically by disinfecting departing planes and ships;
- raising public awareness about this mosquito species and sensitizing the public on preventing its proliferation.

4.3.5 Resistance of An. stephensi to different insecticide classes

An. stephensi mosquitoes that have invaded new geographical areas seem to generally have a genetic background that confers resistance to multiple insecticide classes. This poses potential challenges to its control. However, no data were presented on *An. stephensi*'s susceptibility to chemical classes used in products recently prequalified by WHO (e.g., pyrroles, neonicotinoids). For example, in Jaffna (Sri Lanka), the invaded *An. stephensi* population is highly resistant to DDT (4%), malathion (5%) and deltamethrin (0.05%) *(6)*. In this and other settings, however, it is not clear whether the insecticide resistance detected in the *An. stephensi* population has developed since the vector's invasion or whether the vector arrived with an existing genetic background of insecticide resistance *(6)*.

5. Recommendations to WHO

As demonstrated by the convening of this Technical Consultation, WHO considers the spread of *An. stephensi* to be a major potential threat to malaria control and elimination in Africa and southern Asia. The Technical Consultation recommended that WHO develop a Vector Alert document and post it online to urge WHO Member States and their implementing partners in and around the Horn of Africa, Sudan and the surrounding geographical areas, and Sri Lanka to take immediate action. It was recommended that the Vector Alert summarize the current evidence base on the invasion of *An. stephensi* outlined in section 4 of this report, as well as provide detailed recommendations. The following recommendations were developed by the Technical Consultation with a view to informing WHO communications on the subject:

5.1 What should African countries, especially those in and around the Horn of Africa, do now?

Countries should take the following actions:

- Actively conduct surveillance for *An. stephensi* in urban and peri-urban areas through sampling of its aquatic stages, because methods for collecting adults yield low numbers. Typical breeding sites are human-made containers, particularly water storage containers inside and outside the home, rainwater collections, rooftops, wells, large human-made cisterns, and even clean water ponds. Given the overlap in breeding sites with those of *Aedes spp.*, national malaria control programmes are encouraged to seek close collaboration with the national entities responsible for the control of arboviral vectors, as envisaged in the *Global vector control response 2017–2030 (7).*
- Rear larvae or pupae to adults and identify *An. stephensi* based on the morphological characteristics of the adult female.
- Describe the ecology of *An. stephensi* to help guide control measures.
- Report any new detection of *An. stephensi* to WHO by completing the WHO form to report detection of invasive *Anopheles* vector species and emailing it to vectorsurveillance@who.int. The detection will then be displayed on the Malaria Threats Map.

- If sufficient larvae or pupae are found, these should be reared to adult stage, so that insecticide resistance can be evaluated using WHO susceptibility test procedures. Test results should be reported to WHO alongside data on the occurrence of the vector.
- Specimens should be preserved in Eppendorf tubes on silica gel for molecular analysis, both to confirm the initial morphological identification and to study population dynamics across a recently invaded area. Pinned voucher specimens should also be kept.
- IHR (2005) should be enforced to ensure that any points of entry are free of vectors, to minimize the risk of any further spread of *An. stephensi*.

5.2 What should countries do in areas where the vector has been detected?

Where the vector has been detected, countries should do the following:

- Undertake interventions directed against *An. stephensi*, with the aim of eliminating this species from the invaded areas. This will require an intense effort to enhance and expand the surveillance and control activities currently being implemented.
- Ensure that the immediate focus for the control of *An. stephensi* is on managing vector breeding sites in urban and peri-urban environments. Recommended activities include:
    - removal of breeding sites, where feasible, including filling in of disused wells;
    - modification to prevent vector breeding, including the installation of hermetically sealed lids on water storage containers; and
    - where breeding site removal or modification is not feasible, treatment with WHO-prequalified chemical or biological larvicides, following WHO guidelines.
- Direct local authorities to map remaining breeding sites and inspect them for larval breeding once a week.
- Install mechanical barriers (e.g., window and door screening) to prevent female mosquitoes from entering human dwellings. The aim is to reduce daytime resting opportunities and thus reduce both “dry season mosquito survival” and human exposure.
- Enact or introduce by-laws to regulate water storage practices and construction work so as to avoid the creation of potential breeding sites.
- Enforce IHR (2005) to ensure that airports and other points of exit are free of vectors. Treat departing aircraft and ships to remove insects, following WHO guidance.
- Raise public awareness of this mosquito species, the aim being to contribute to reducing breeding sites and preventing the proliferation of the vector.

5.3 How should interventions be monitored and evaluated?

To monitor and evaluate, countries should do the following:

- Given that experience with *An. stephensi* in Africa is limited or absent, base monitoring and evaluation activities on best practices drawn from experience gained in areas of the vector’s original distribution (e.g., in India and Iran, as described above). An active effort should be made to build an evidence base to inform the control and elimination of this vector in Africa.
- To evaluate the effectiveness of antilarval interventions, monitor the presence or absence of *An. stephensi* larvae in identified breeding sites.

- To evaluate the effectiveness of regulation and activities designed to reduce the number of urban and peri-urban breeding sites, or their suitability for mosquito breeding, survey these areas for the creation or presence of suitable breeding sites.

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Technical consultation on the spread of *Anopheles stephensi*

Malaria Policy Advisory Committee Meeting
Geneva, Switzerland
2 – 4 OCTOBER 2019

Global **Malaria** Programme

World Health Organization

Anopheles stephensi

- three ecological variants; type, intermediate and mysorensis
- ‘type’ form is an efficient urban malaria vector in India due to its anthropophilic nature and adaptation to man-made breeding sites
- ‘type’ and ‘intermediate’ forms have also emerged as efficient vectors in rural areas of India as a result of changing agricultural and water storage practices
- quickly adapt to the local environment & withstands high temperatures
- an efficient urban malaria vector for both *Plasmodium falciparum* and *P. vivax*
- until 2011, the reported distribution was confined to certain countries in South-East Asia and large parts of the Arabian Peninsula

An. stephensi breeding sites (Ethiopia)

Why hold a technical consultation?

Acta Tropica 139 (2014) 39–43

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

First record of the Asian malaria vector *Anopheles stephensi* and its possible role in the re

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DOI 10.1186/s12936-017-1977-7

Malaria Journal

CASE REPORT Open Access

First record of *Anopheles stephensi* in Sri Lanka: a pote of malaria rei

A. G. Gayan Dharmasiri[1], A. Yasl
Kandasamy Aravindan[2], H. T. R.

Acta Tropica 188 (2018) 180–186

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

First detection of *Anopheles stephensi* Liston, 1901 (Diptera: culicidae) in Ethiopia using molecular and morphological approaches

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Technical Consultation Objectives (1)

- Review published and unpublished evidence on the presence of *An. stephensi* outside of its traditional geographic range;
- Review efforts to model potential areas at risk for *An. stephensi* introduction and assess – to the extent feasible – the potential risk for further spread beyond the vector's previously reported geographic range;
- Review knowledge on *An. stephensi's* bionomics and biology, and analyse differences between vector populations in Asia and Africa to identify suitable control practices for each area where the vector is present;

Technical Consultation Objectives (2)

- Review countries' experiences of controlling *An. stephensi* where it has been traditionally present, with the aim of identifying best practices and the main challenges in the control of this species;
- Review the status of *An. stephensi* resistance to different insecticide classes;
- Recommend surveillance and control strategies to address the threat posed by the spread of *An. stephensi* and surveillance indicators to assess the impact of control interventions.

Broad Participation

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Conclusions

- *An. stephensi* has been spreading over the last decades
- Djibouti, Sri Lanka and Ethiopia were only the most recently affected countries

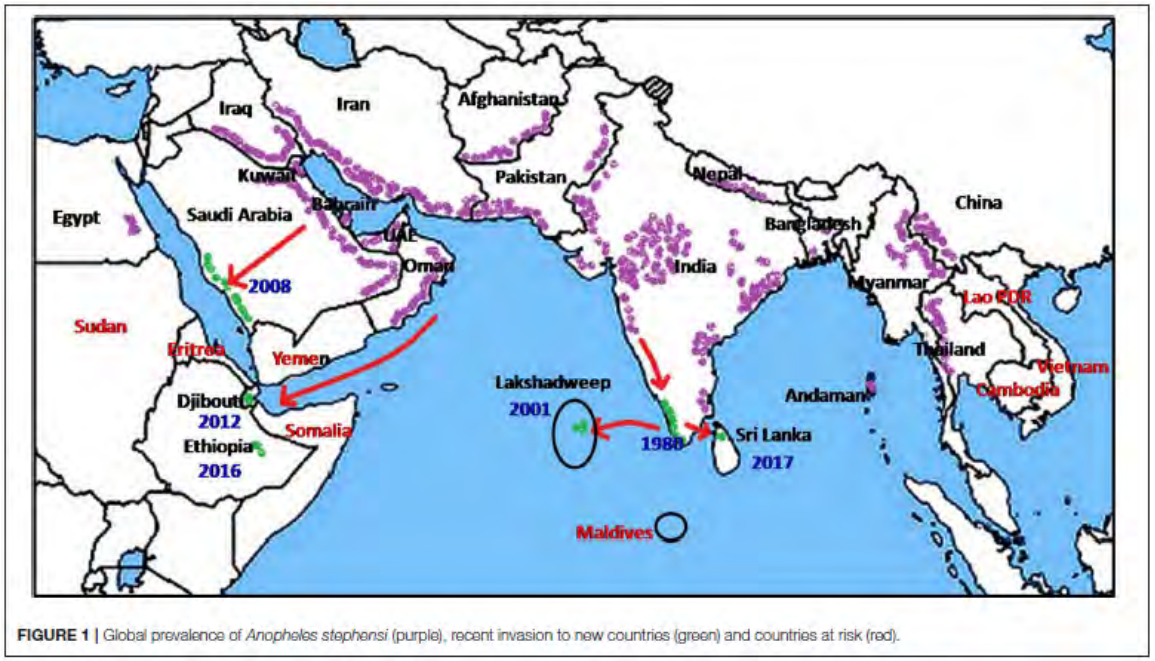


FIGURE 1 | Global prevalence of *Anopheles stephensi* (purple), recent invasion to new countries (green) and countries at risk (red).

- Sudan has since joined the list (Ayman Ahmed, per. Com.)
- Further spread must be anticipated (or has already occurred)

Figure from Surendran *et al. (*2019) Anthropogenic Factors Driving Recent Range Expansion of the Malaria Vector *Anopheles stephensi*. Front. Public Health 7:53.

Conclusions

- Evidence of actual or potential for transmission of both *P. falciparum* and *P. vivax* in Djibouti and Ethiopia
- Experiences of controlling *An. stephensi* in Africa is limited or absent. Surveillance and control approaches should thus be based on best-practices from India until context specific experience has been developed.
- *An. stephensi* mosquitoes that invaded new geographical areas generally have a genetic background that confers resistance to multiple insecticide classes, posing potential control challenges. However, no data on susceptibility to pyrroles or neonicotinoids were reviewed.
- New tools for surveillance and control need development and evaluation, including – once available – a self-limiting *An. stephensi* gene-drive construct that aims to produce non-biting male mosquitoes to suppress local wild populations
- Model-based assessments of mosquito threats need further development, incl. on key variables and how to collect/incorporate these

Recommendations to WHO

- Develop a ‘Vector Alert’ document and post it online to urge WHO Member States and their implementing partners in and around the Horn of Africa, Sudan and the surrounding geographical areas, and Sri Lanka to take immediate action

- Action in three areas:
  - Surveillance (including updates to mosquito identification keys)
  - Intervention
  - Monitoring & evaluation

- Develop data reporting sheet

- Update Malaria Threats Map to illustrate current and new reports of *An. stephensi* distribution / invasion (allowing potential expansion to report other invasive anopheline species)

What has happened since?

Global **Malaria** Programme

Vector alert: *Anopheles stephensi* invasion and spread

Horn of Africa, the Republic of the Sudan and surrounding geographical areas, and Sri Lanka

AUGUST 2019

INFORMATION NOTE

IDENTIFIED THREAT

Anopheles stephensi, a highly competent vector of *Plasmodium falciparum* and *P. vivax*, is considered an efficient vector of urban malaria. In parts of India, two biological forms of *An. stephensi* – "type" and "intermediate" – have also emerged as efficient vectors in rural areas, due to changing agricultural and water storage practices and urbanization. The third form – "mysorensis" – is considered to be a poor vector, although it has been involved in malaria transmission in certain rural areas in Afghanistan and Iran. Until 2011, the reported distribution of *An. stephensi* was confined to certain countries in South-East Asia and large parts of the Arabian Peninsula. Since then, the vector has been reported from Djibouti (2012), Ethiopia (2016), Sri Lanka (2017) and most recently from the Republic of the Sudan (2019). In the Horn of Africa, the vector seems to be spreading from its first site of detection (Djibouti) to neighbouring countries. *An. stephensi* typically breeds in containers or cisterns with clean water, and the vector appears to quickly adapt itself to the local environment (including cryptic habitats such as deep wells); it also survives extremely high temperatures during the dry season, when malaria transmission usually reaches a seasonal low. In addition, the genetic background of introduced *An. stephensi* seems to confer resistance to multiple insecticide classes, posing potential challenges to its control.

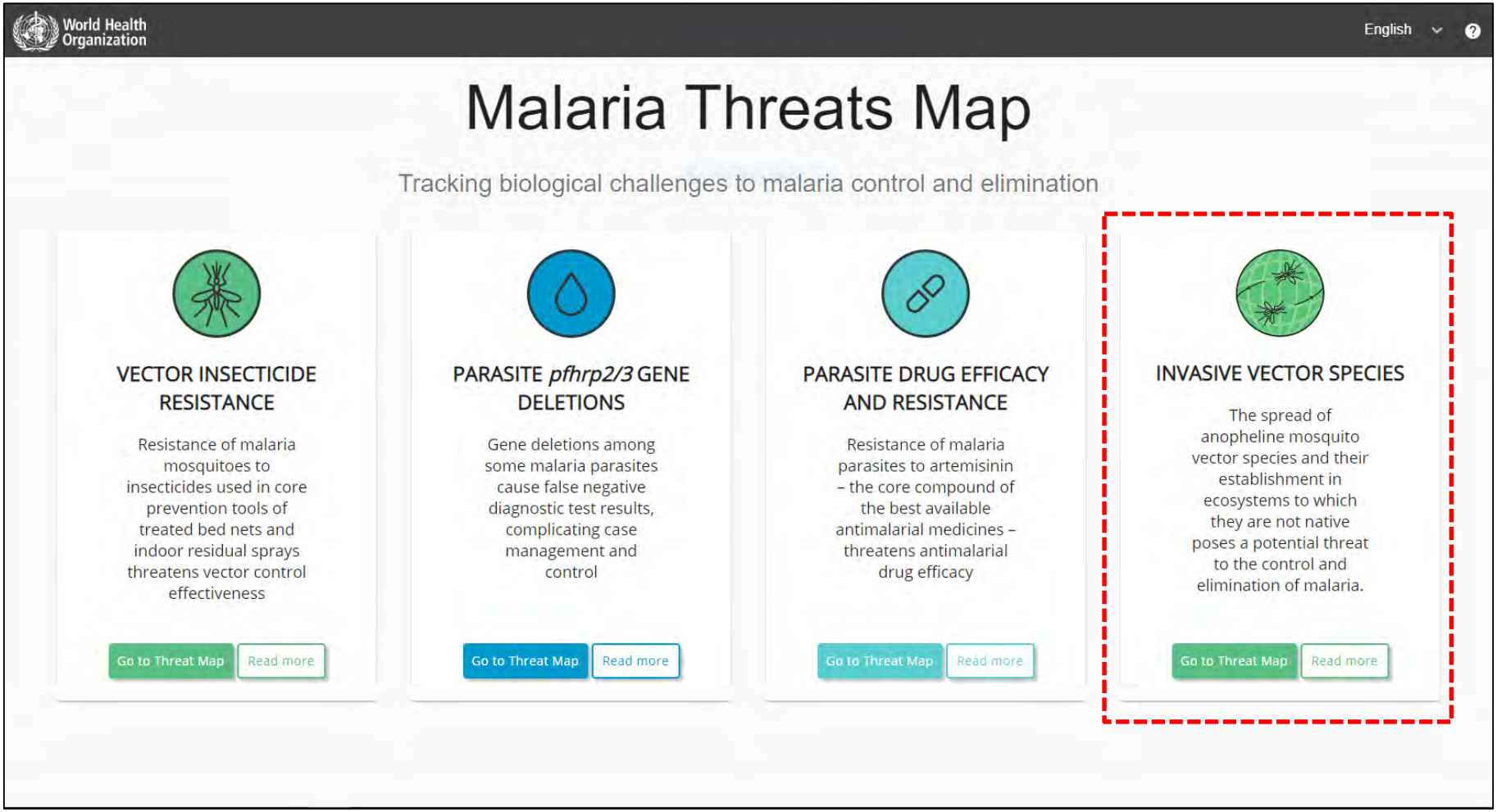
The World Health Organization (WHO) considers the spread of *An. stephensi* to be a major potential threat to malaria control and elimination in Africa and southern Asia. This vector alert has been developed to urge WHO Member States and their implementing partners – especially those in and around the Horn of Africa, the Republic of the Sudan and surrounding geographical areas, and in Sri Lanka – to take immediate action, as outlined below.

World Health Organization

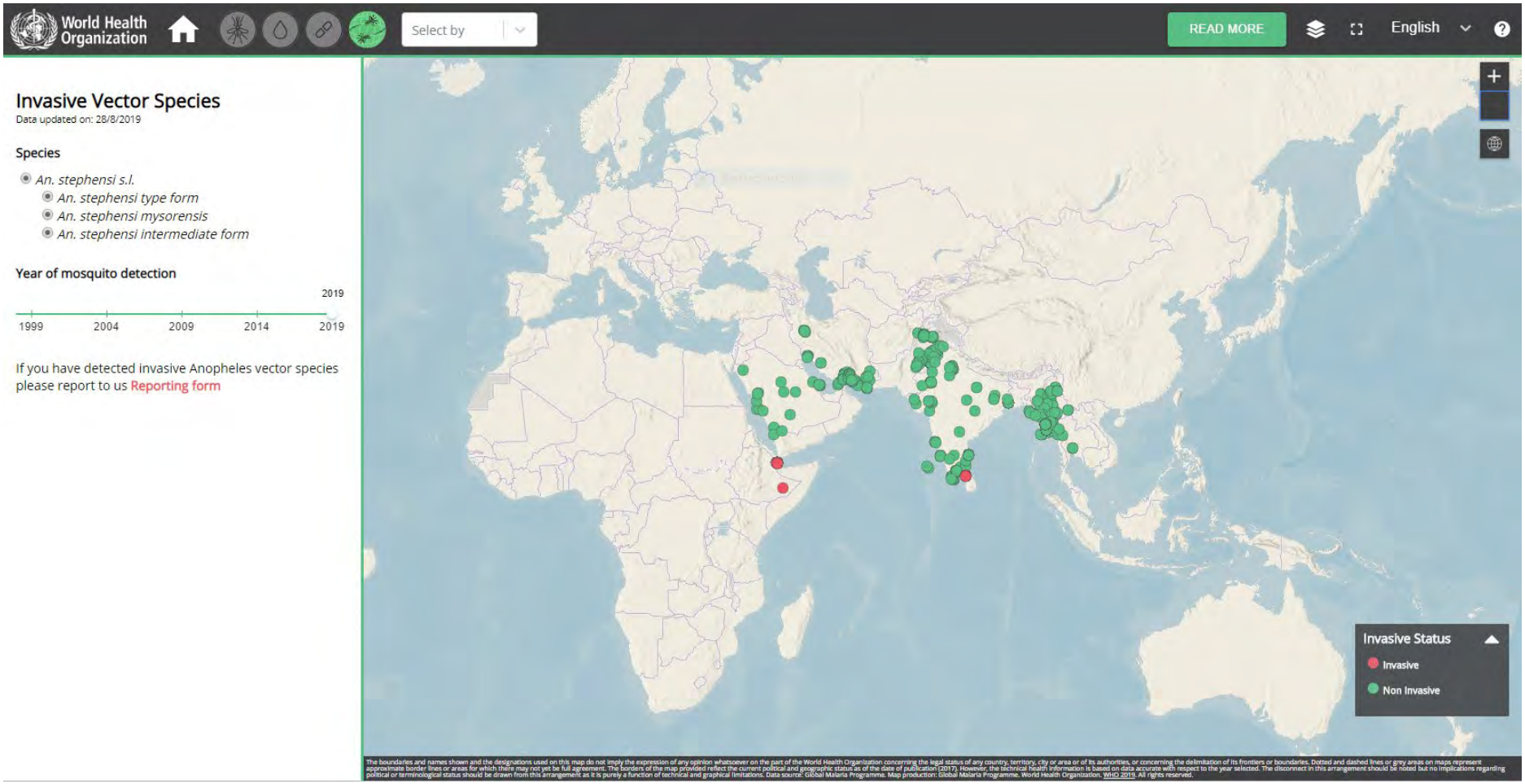
English & French versions online. Arabic undergoing layout.

Accompanied by data reporting form and new email account for data reporting

What has happened since?



What has happened since?



Reporting new detections:

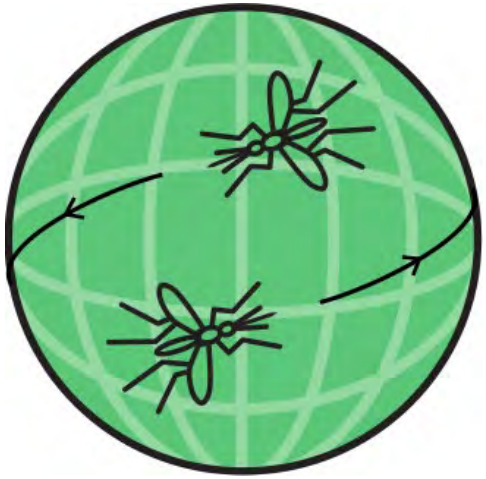
Form: https://web-prod.who.int/docs/default-source/documents/publications/gmp/whogmp-invasive-species-reporting-form.xlsm?sfvrsn=8c82af32_21

Send to: vectorsurveillance@who.int

Global **Malaria** Programme

World Health Organization

Further Information



https://www.who.int/publications-detail/vector-alert-anopheles-stephensi-invasion-and-spread

https://www.who.int/fr/news-room/detail/26-08-2019-vector-alert-anopheles-stephensi-invasion-and-spread

Malaria Policy Advisory Committee Meeting
2–4 October 2019, Geneva, Switzerland
Background document for Session 7

Updating the WHO G6PD classification of variants and the International Classification of Diseases, 11th Revision (ICD-11)

October 2019

Background and rationale

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked recessive disorder. It is the most common genetic abnormality affecting an estimated 400 million people worldwide. Although mostly asymptomatic, G6PD deficiency can lead to three clinical manifestations: (i) neonatal jaundice; (ii) acute haemolytic anaemia (AHA) triggered by infection, drugs (such as 8-aminoquinolines, e.g., primaquine/tafenoquine) or fava beans; and (iii) chronic non-spherocytic haemolytic disease (CNSHD). The gene encoding for G6PD is highly polymorphic, with over 300 variants and variable phenotypic expression in heterozygous females, depending on X-inactivation patterns. Variants are also associated with variable haemolytic risk, but this is not well characterized. G6PD deficiency is more common in malaria-endemic countries, and there is some evidence that the heterozygous state (females) confers protection from *Plasmodium* infection.

In the first-ever international WHO meeting on G6PD, held in December 1966, just 20 G6PD variants were described according to their biochemical characteristics, such as percentage (%) activity (measured by gold standard spectrophotometric assay), electrophoretic mobility, Km value, pH optimum and thermostability. This format served as the template for many publications over the next 20 years *(1).* The report from the meeting also recommended that the name of any G6PD variant be followed by an indicator of its activity, as follows: "(-) indicates 25% or less activity; (+/-) indicates 25–65% activity; (+) indicates normal activity (65–150%); (++) indicates greater than 150% activity". However, it was not until five years later that Yoshida, Beutler and Motulsky proposed the classification scheme we are familiar with today (Table 1), in an article published in the Bulletin of the World Health Organization *(2).* This classification scheme was accompanied by cautionary statements that have received less attention. These include: "*for purposes of convenience, the variants described in the accompanying table are somewhat arbitrarily divided into five classes*", and "*the distinction between these classes is not always clear*". Yoshida et al.'s classification quickly became known as the "WHO classification", even though the authors did not claim to have any mandate from WHO.

Table 1. Yoshida et al.'s proposed classification of G6PD, known as the "WHO Classification (Class I–V)", 1971

I. Activity <10% of normal, severe enzyme deficiency with chronic non-spherocytic haemolytic anaemia (CNSHA)

II. Activity <10% of normal, severe enzyme deficiency

This document was prepared as a pre-read for the meeting of the Malaria Policy Advisory Committee and is not an official document of the World Health Organization.
WHO/CDS/GMP/MPAC/2019.15

III. Activity 10–60% of normal, moderate to mild enzyme deficiency, intermittent acute haemolysis

IV. Very mild or no enzyme deficiency (60–100% of normal)

V. Increased enzyme activity (more than twice normal)

Yoshida et al.'s 1971 classification scheme was further reinforced when WHO assembled a Working Group on G6PD deficiency in 1985. The meeting report in the Bulletin of the World Health Organization (*3*) described 310 G6PD variants according to a slightly modified version[1] of the five classes in Table 1. Since 1986, when the full G6PD cDNA sequence was published, it has been possible to identify the individual mutations underlying many of the G6PD variants already known. Despite no formal WHO recommendation for change, over the past 30 years, some reports of new variants have provided both biochemical characterization and the identity of the underlying mutation(s). There has been a gradual shift from biochemical analysis to mutation analysis. However, biochemical data are important because they explain how the enzyme operates, which, in turn, influences response to oxidative stress.

In addition, technical consultations to inform the performance requirements for G6PD point-of-care tests to guide treatment with primaquine and tafenoquine have redefined 'normal' G6PD activity to be either >70% or >80% of normal *(4,5)*. No single additional case of G6PD *Hektoen* with activity >150% has ever been described, and some forms of CNSHA may have G6PD activity >10%. Most crucially, the cut-off of 10% activity that separates class II from class III was from its inception completely arbitrary, and a large number of G6PD variants currently classified as class II and class III have the same clinical manifestations. Furthermore, although certain variants that are currently in class II (e.g., G6PD Mediterranean), on average, yield more severe clinical manifestations than certain class III variants (e.g., G6PD A-), there is extensive overlap, and AHA can be severe or even life-threatening with any class II or class III variant. Finally, many variants have been placed into class II or class III based on a single measurement in a single person. Consensus is therefore needed on the process for determining the activity of a specific variant, e.g., number of patients, number of repeats. For these reasons, we propose that a Technical Consultation be held to revise the G6PD classification scheme and simultaneously incorporate these findings into a proposal to modify ICD-11[2]. ICD-11 currently only classifies G6PD deficiency under haemolytic anaemias, specifically as haemolytic anaemia due to G6PD deficiency (code: 3A10.00[3]). The current version of ICD-11 (04/19) will be updated in February 2020. Therefore, there is some urgency to submit a proposal to expand upon this classification so as to better reflect the occurrence of this condition, as well as the severity and range of clinical manifestations.

Why should the Global Malaria Programme (GMP) lead this effort?

In the malaria treatment guidelines *(6)*, WHO recommends that G6PD status be known prior to administration of primaquine. Therefore, scale-up of safe and effective radical cure for *P. vivax* is dependent upon the corresponding availability of quality, point-of-care G6PD testing and expansion of our knowledge on the haemolytic risk of various G6PD variants. These efforts should be

[1] Class I – associated with chronic non-spherocytic haemolytic anaemia (CNSHA). Class II – severely deficient: less than 10% residual activity. Class III – moderately deficient: 10–60% residual activity. Class IV – normal activity: 60–150%. Class V – increased activity.

[2] https://icd.who.int/en/

[3] ICD-11: 3A10.00 – Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common hereditary erythrocyte enzyme deficiency that can manifest with severe neonatal jaundice which can lead to serious neurological consequences, or, most often, with acute haemolytic anaemia following ingestion of certain foods (fava beans), common drugs (some antimalaria drugs, sulphamides, analgesics), or in the course of an infection, in otherwise asymptomatic individuals.

underpinned by updated nomenclature (also included in ICD-11) that can inform future monitoring of prevalence and clinical manifestations, and product development.

Over the past year, GMP, Prevention, Diagnosis and Treatment unit staff have attempted to identify the relevant departments/units of WHO responsible for guidance on G6PD. The Hereditary Diseases Programme/Human Genetics Programme that convened the consultations in the 1980s no longer exists, and G6PD deficiency seems to have been somewhat orphaned during the reorganizations over the past decades. Fortunately, the WHO Genomics Initiative, now hosted by the WHO Department of Service Delivery and Safety, convened an expert meeting in April 2019 on genomics and genetic disorders. The goals of the meeting were to set priority areas of work in low- and middle-income countries and develop a roadmap on genetics and genomics. Revising the current G6PD classification scheme was identified as a priority to take forward. Subsequent internal discussions with the ICD team revealed that G6PD is also under-represented in the current version, but that new modifications to the ICD architecture will enable a much more detailed categorization. Thus, the revisions to both the classification and ICD catalogue could be accomplished in parallel. The WHO Department of Service Delivery and Safety proposed that GMP coordinate the consultations required to achieve these revised schemes, as the results will immediately impact our work towards establishing policy and product specifications for point-of-care G6PD tests to guide use of 8-aminoquinolines for radical cure of *P. vivax.*

Objectives

i) Revise the most widely used classification of G6PD variants.

ii) Discuss requirements for defining new variants.

iii) Propose new categorization of G6PD for ICD-11, including classification of G6PD variants and clinical manifestations.

References

1. Standardization of procedures for the study of glucose-6-phosphate dehydrogenase, WHO Technical Report Series, no. 366. Geneva: World Health Organization; 1967 (https://apps.who.int/iris/bitstream/handle/10665/40660/WHO_TRS_366.pdf?sequence=1&isAllowed=y).
2. Yoshida A, Beutler E, Motulsky AG. Human glucose-6-phosphate dehydrogenase variants. Bull World Health Organ. 1971;45(2):243–53.
3. Glucose-6-phosphate dehydrogenase deficiency. WHO Working Group. Bull World Health Organ. 1989;67:601–11.
4. Point-of-care G6PD testing to support safe use of primaquine for the treatment of vivax malaria. WHO Evidence Review Group meeting report 8–9 October 2014. Geneva: World Health Organization; 2014 (https://www.who.int/malaria/mpac/mpac-march2015-erg-g6pd.pdf).
5. Lacerda MVG, Llanos-Cuentas A, Krudsood S, Lon C, Saunders DL, Mohammed R, et al. Single-dose tafenoquine to prevent relapse of *Plasmodium vivax* malaria. N Engl J Med. 2019;380:215–28. doi:10.1056/NEJMoa1710775.
6. Guidelines for the treatment of malaria. Third edition. Geneva: World Health Organization; 2015 (https://www.who.int/malaria/publications/atoz/9789241549127/en/).

Revision of WHO classification of G6PD variants and International classification of diseases (ICD)-11

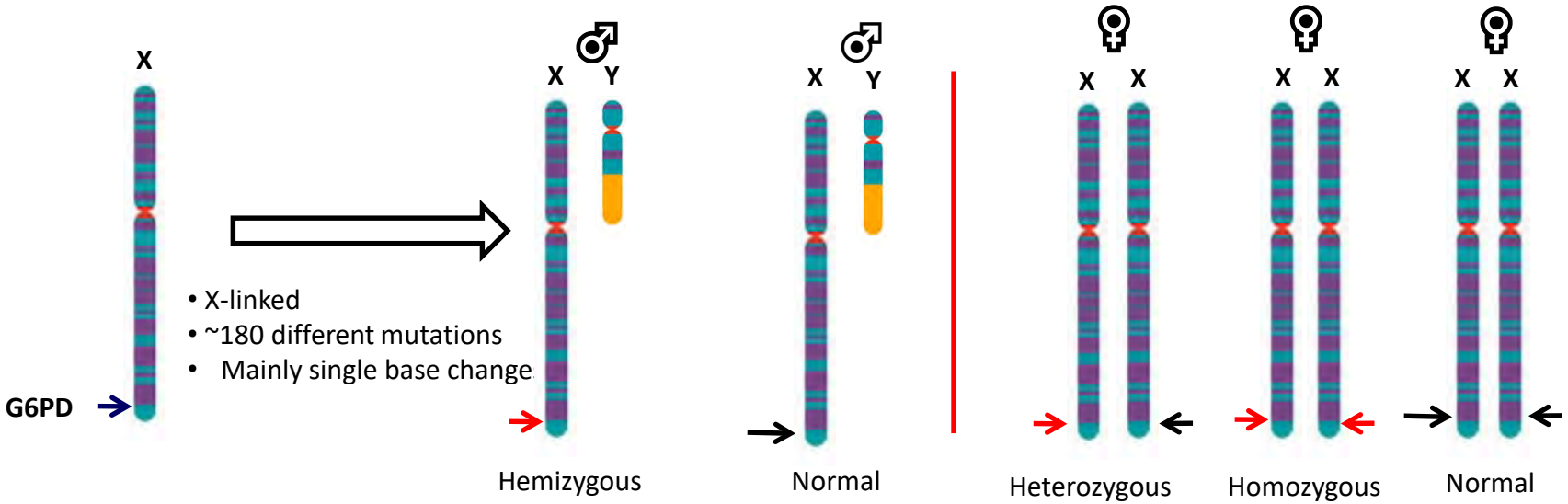
Dr J. Cunningham and Dr. A. Bosman
Prevention, Diagnostics and Treatment

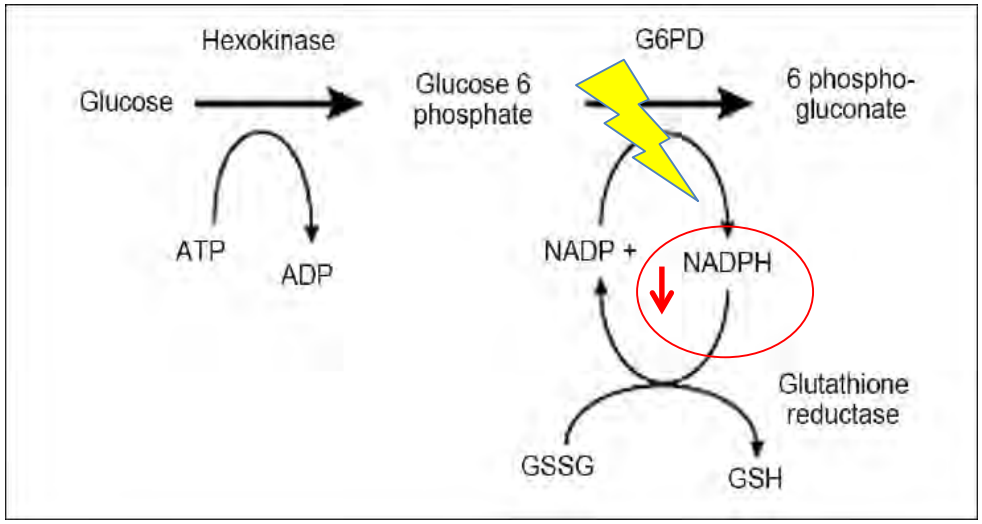
Global **Malaria** Programme

World Health Organization

What is G6PD deficiency ?

- X-linked, hereditary genetic defect due to mutations in the *G6PD* gene, causing functional variants with many biochemical and clinical phenotypes





Drugs like 8 aminoquninolones create oxidative metabolites

Factors that can affect G6PD activity:

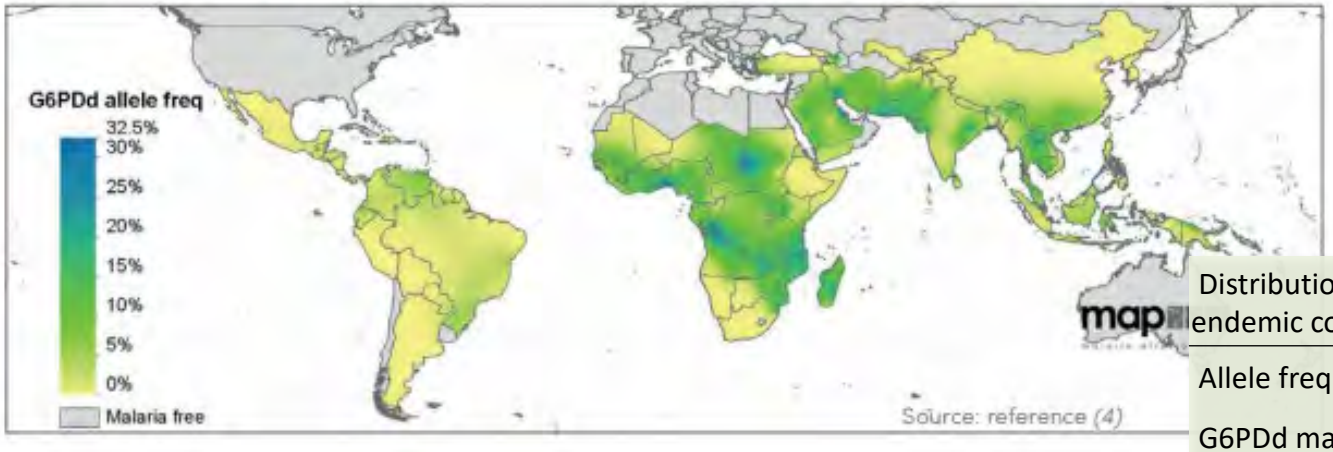
- G6PD variant – mutations variable stability
- Age of RBCs – older RBC more vulnerable
- Anaemia (malaria/Fe def)
- Hemoglobinopathies reducing RBC survival
- Reticulocytes resistance to oxidative stress

World Health Organization

G6PD deficiency and *P. vivax* malaria

Prevalence of G6PD deficiency

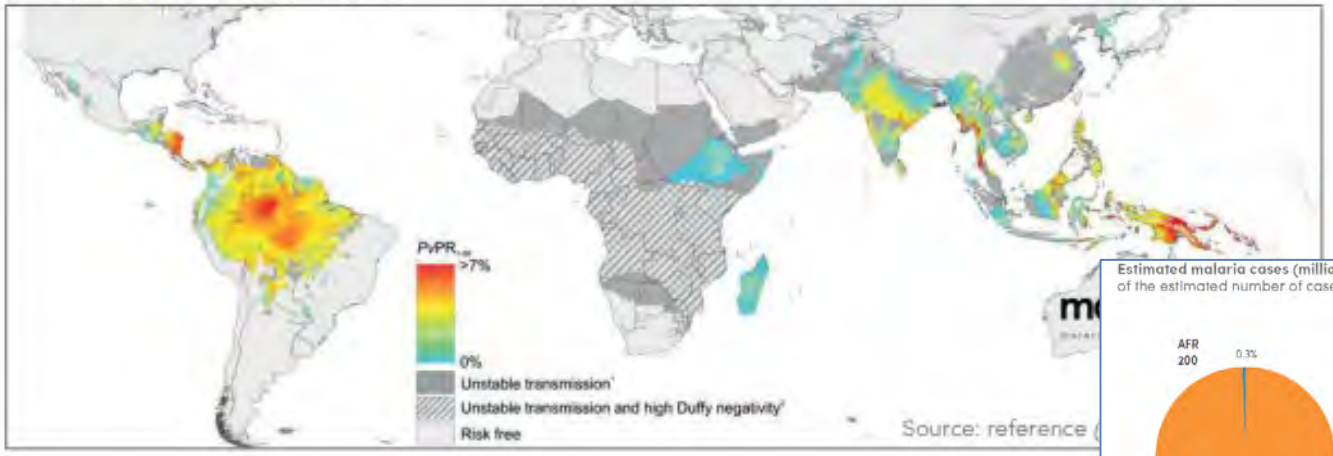
~ 400 million people affected

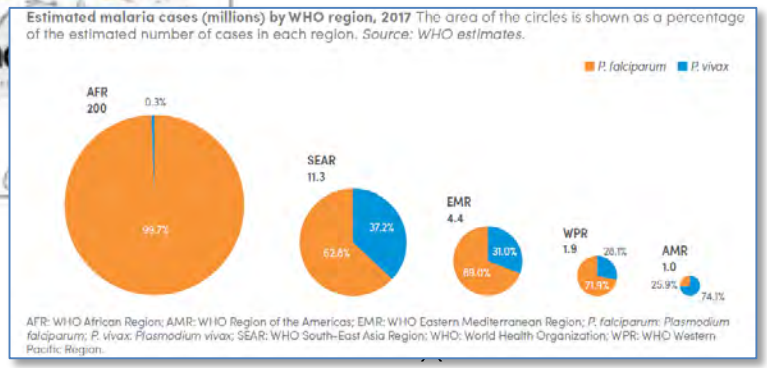


Distribution in endemic countries	Q25	**Median**	Q75
Allele frequency	7.44%	**8.04%**	8.81%
G6PDd males	203,729	**220,130**	241,114
G6PDd females*	121,618	**132,932**	147,814

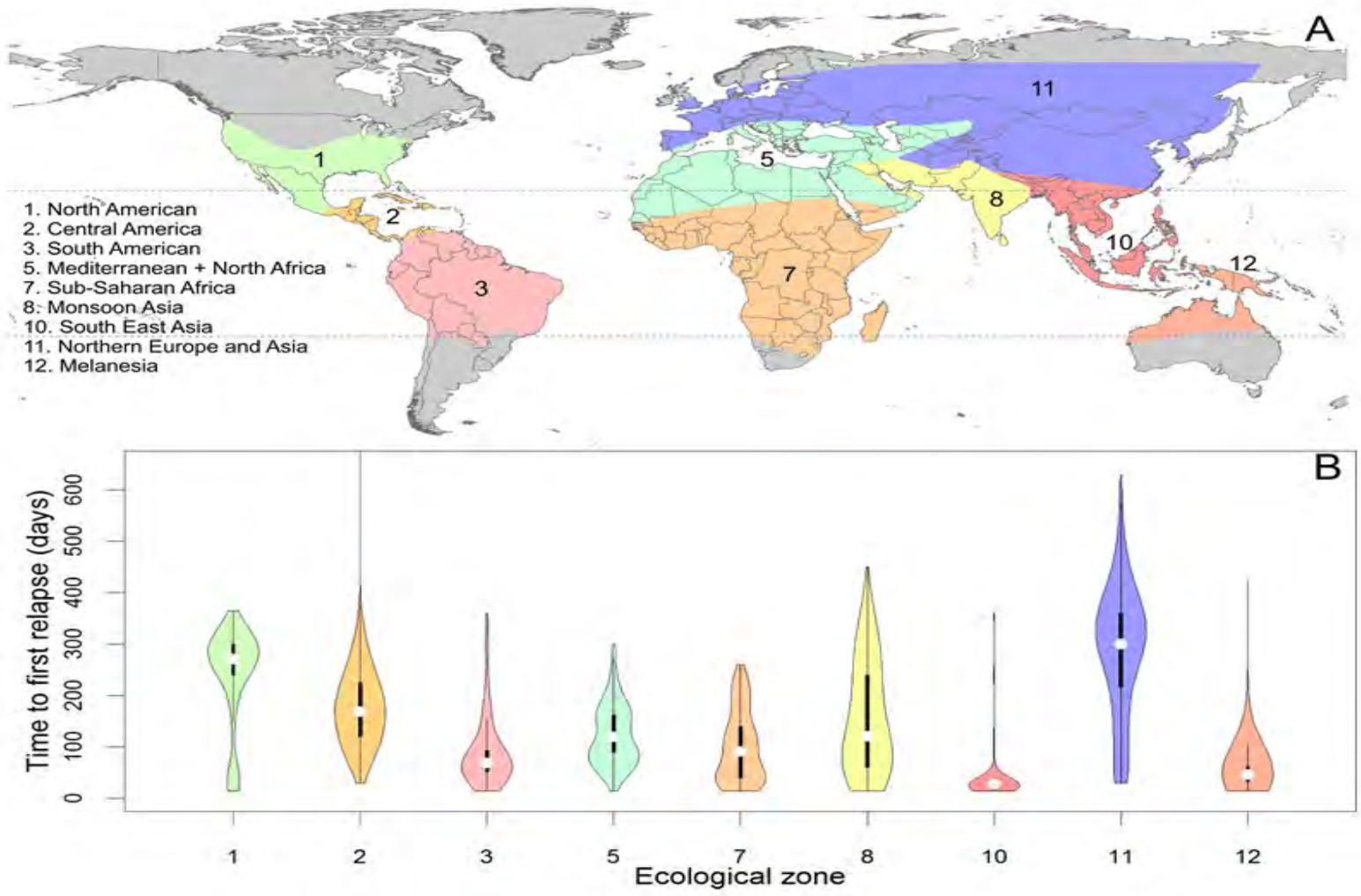
FIGURE 2
Endemicity of *P. vivax* in 2010

~ 7.5 million estimated in 2017





Relapses and vivax transmission



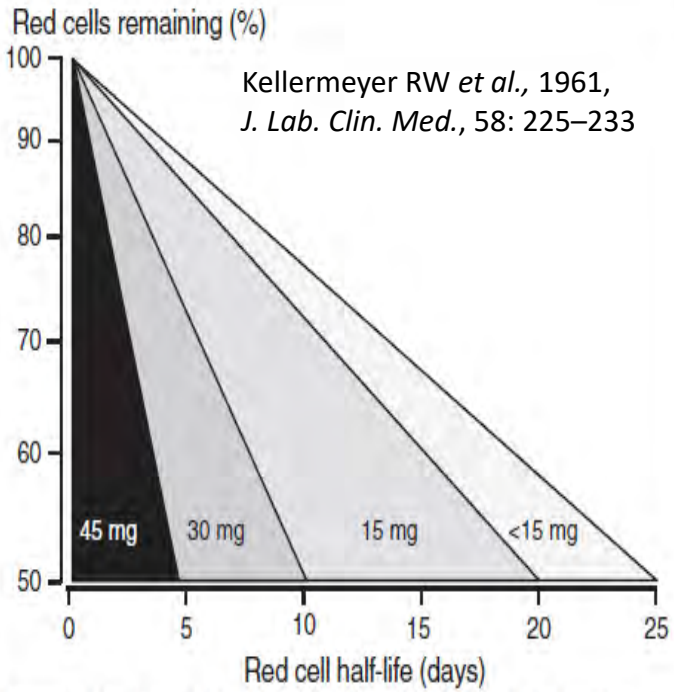
Battle KE *et a*l., *Adv Parasitol 2012; 80*:1-111

8-aminoquinolines and G6PD deficiency

- Individual and public health risks posed by relapsing *P. vivax* should be taken into account when considering the risks and benefits of anti-relapse treatment

Urine collection of a 5-year-old child with G6PD deficiency on D4, D5 and D6 (from left to right) after the 4th daily dose of primaquine 15mg.
At admission to the pediatric emergency hospital of Wad Medani, the child had Hb at 2 g/dL, corrected to 8 g/dL after blood transfusion (Dec 2018)

Primaquine dose dependent hemolysis



Kellermeyer RW *et al.,* 1961, *J. Lab. Clin. Med.*, 58: 225–233

Figure 4 Studies of ^{51}Cr labelled red cell survival in healthy adult hemizygous male volunteers with the A-variant of G6PD deficiency exposed to different dose regimens of primaquine in studies conducted by the University of Chicago-Army Medical Research Unit at the Illinois State penitentiary (Stateville) from 1950 to 1962. Daily doses are shown within the range of red cell survivals that resulted. Daily administration of 45 mg base primaquine was considered to result in "dangerous haemolytic anaemia", daily administration of 30 mg resulted in severe haemolysis and acute anaemia, and daily administration of 15 mg resulted in moderate haemolysis and mild anaemia [36].

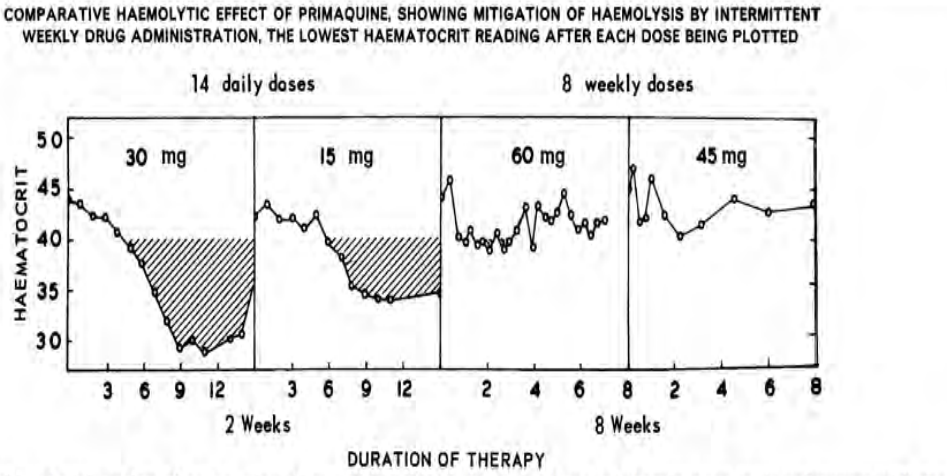


Figure 5 Once weekly administration of primaquine 45 mg base for 8 weeks to healthy adult hemizygous male volunteers with the A- variant of G6PD deficiency resulted in less anaemia than daily administration of 15 mg for two weeks [35].

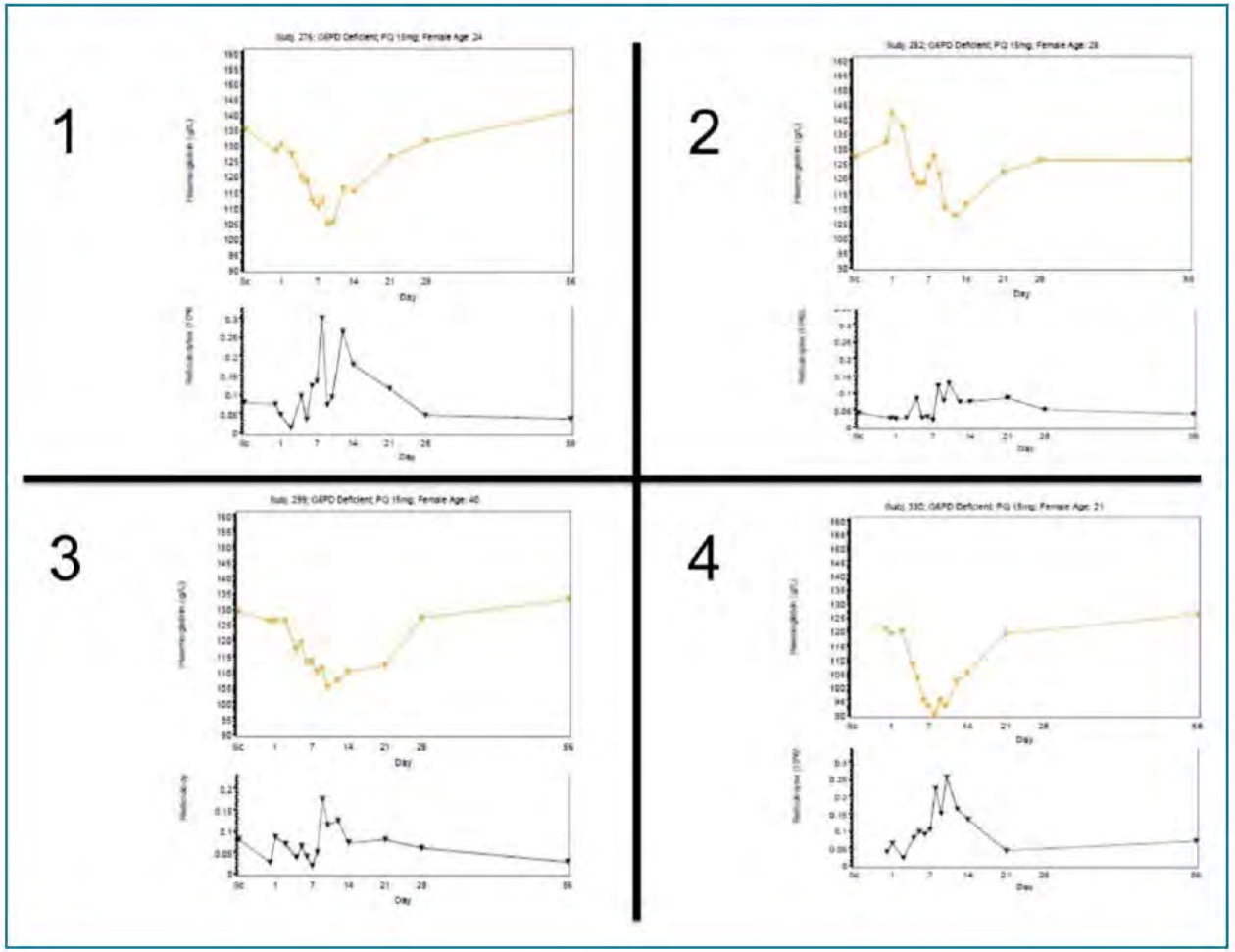
Alving AS *et al.* 1960, *Bulletin of WHO*, *22:* 621–631

Haemolytic response following daily and weekly doses of primaquine in the same subject, a male volunteer with A- variant of G6PD deficiency

World Health Organization

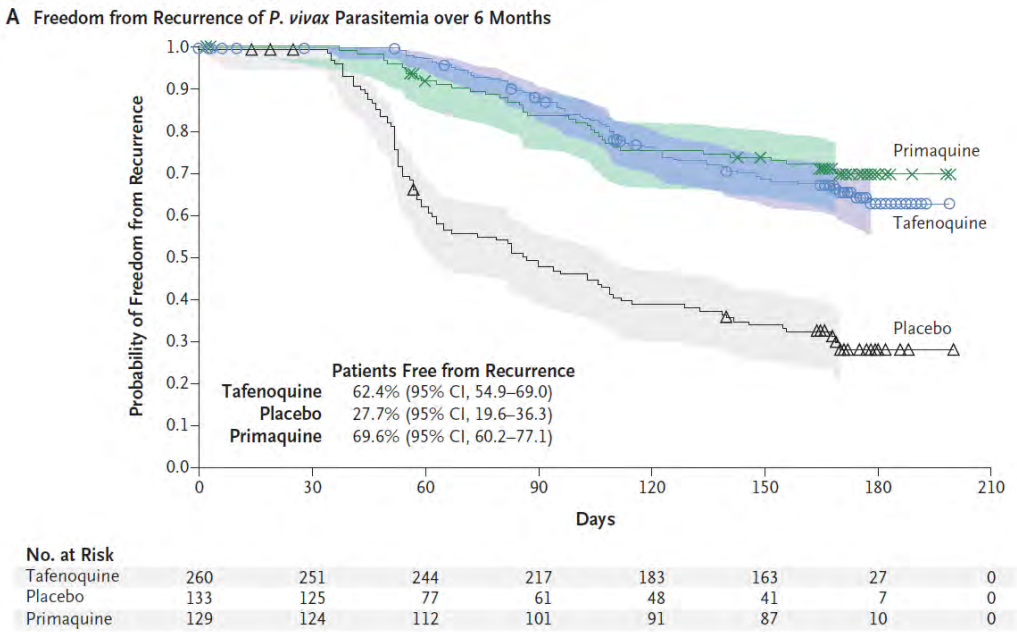
Response to PQ in female heterozygous

- In a GSK-sponsored study of tafenoquine (TAF 110027), 4 heterozygous women were treated with 15 mg primaquine base for 14 days and showed a level of drop of Hb (2.5 g/dL) similar to that observed in all patients with G6PD deficiency. These women had G6PD activity levels ranging between 40% and 60% of normal.



Haemoglobin (orange, above) and reticulocyte (black, below) levels following daily primaquine for 14 days at 0.25 mg/kg/day among four women heterozygous for G6PD deficiency - Courtesy of GSK.

Tafenoquine versus primaquine trials



Patients were assigned to receive tafenoquine (single 300-mg dose), placebo, or primaquine (15 mg, administered once daily for 14 days) in addition to a 3-day course of chloroquine (total dose of 1500 mg).

Lacerda *et al.* N Engl J Med 2019; 380: 215-227

Krintafel® (tafenoquine) prescribing information:

- Contraindication: G6PD deficiency or unknown G6PD status
- Patients were excluded from clinical trials of Krintafel if they had a G6PD enzyme activity level <70% of the site median value for G6PD normal activity

https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/210795s000lbl.pdf

New point of care G6PD diagnostics

Point-of-care tests for G6PD deficiency

- Quantitative read-out; analyzer, multi-steps
- adjustment for Hb and temperature
- Required for tafenoquine

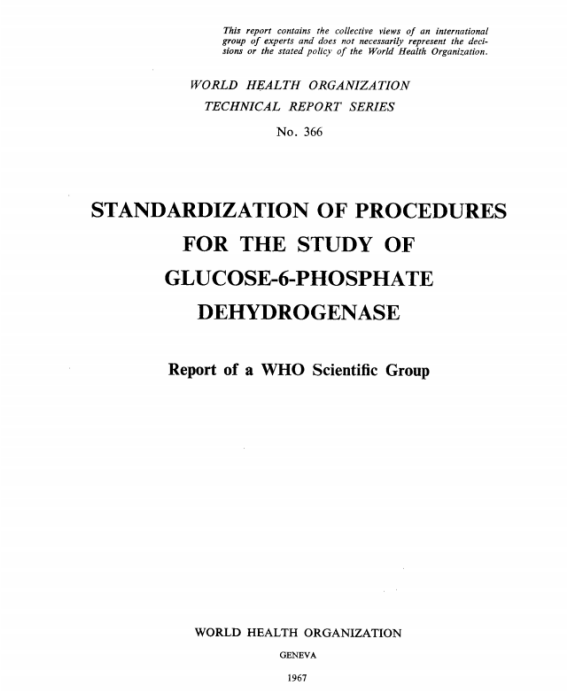
- RDT-like format
- Discriminate < and > 30% activity (ok for males); subjective read-out
- No adjustment for Hb and temperature

Common features

- Finger prick blood, result in < 10 mins
- 12 month shelf life
- Storage 25-30°C

Brief History of G6PD Classification

- WHO scientific Group – 1967
- Yoshida et al - Bulletin of WHO - 1971
- WHO working group 1985 - Published 1989 in Bulletin of WHO



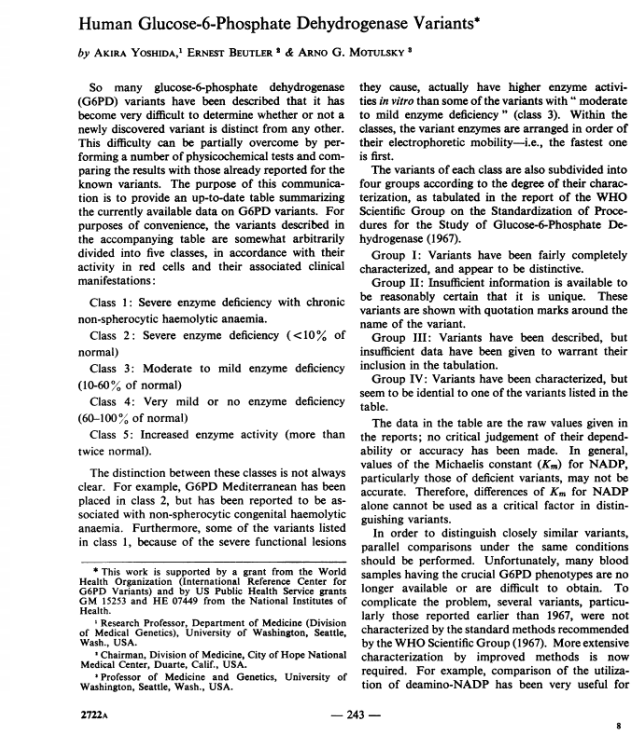
This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES
No. 366

STANDARDIZATION OF PROCEDURES FOR THE STUDY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE

Report of a WHO Scientific Group

WORLD HEALTH ORGANIZATION
GENEVA
1967



Human Glucose-6-Phosphate Dehydrogenase Variants*

by AKIRA YOSHIDA,[1] ERNEST BEUTLER [2] & ARNO G. MOTULSKY [3]

So many glucose-6-phosphate dehydrogenase (G6PD) variants have been described that it has become very difficult to determine whether or not a newly discovered variant is distinct from any other. This difficulty can be partially overcome by performing a number of physicochemical tests and comparing the results with those already reported for the known variants. The purpose of this communication is to provide an up-to-date table summarizing the currently available data on G6PD variants. For purposes of convenience, the variants described in the accompanying table are somewhat arbitrarily divided into five classes, in accordance with their activity in red cells and their associated clinical manifestations:

Class 1: Severe enzyme deficiency with chronic non-spherocytic haemolytic anaemia.

Class 2: Severe enzyme deficiency (<10% of normal)

Class 3: Moderate to mild enzyme deficiency (10-60% of normal)

Class 4: Very mild or no enzyme deficiency (60–100% of normal)

Class 5: Increased enzyme activity (more than twice normal).

The distinction between these classes is not always clear. For example, G6PD Mediterranean has been placed in class 2, but has been reported to be associated with non-spherocytic congenital haemolytic anaemia. Furthermore, some of the variants listed in class 1, because of the severe functional lesions they cause, actually have higher enzyme activities *in vitro* than some of the variants with " moderate to mild enzyme deficiency " (class 3). Within the classes, the variant enzymes are arranged in order of their electrophoretic mobility—i.e., the fastest one is first.

The variants of each class are also subdivided into four groups according to the degree of their characterization, as tabulated in the report of the WHO Scientific Group on the Standardization of Procedures for the Study of Glucose-6-Phosphate Dehydrogenase (1967).

Group I: Variants have been fairly completely characterized, and appear to be distinctive.

Group II: Insufficient information is available to be reasonably certain that it is unique. These variants are shown with quotation marks around the name of the variant.

Group III: Variants have been described, but insufficient data have been given to warrant their inclusion in the tabulation.

Group IV: Variants have been characterized, but seem to be identical to one of the variants listed in the table.

The data in the table are the raw values given in the reports; no critical judgement of their dependability or accuracy has been made. In general, values of the Michaelis constant (K_m) for NADP, particularly those of deficient variants, may not be accurate. Therefore, differences of K_m for NADP alone cannot be used as a critical factor in distinguishing variants.

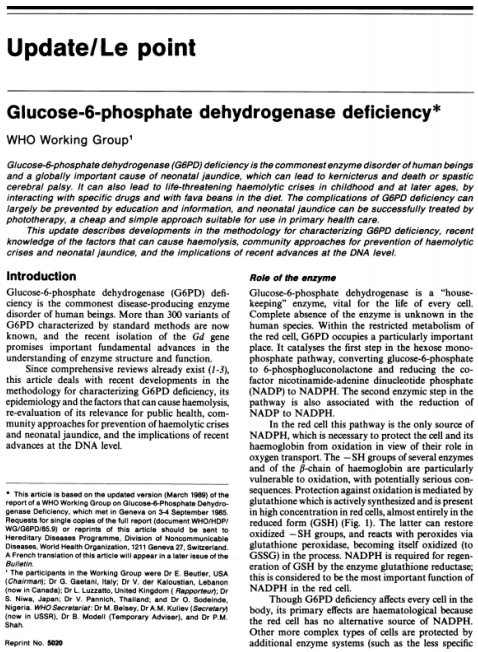
In order to distinguish closely similar variants, parallel comparisons under the same conditions should be performed. Unfortunately, many blood samples having the crucial G6PD phenotypes are no longer available or are difficult to obtain. To complicate the problem, several variants, particularly those reported earlier than 1967, were not characterized by the standard methods recommended by the WHO Scientific Group (1967). More extensive characterization by improved methods is now required. For example, comparison of the utilization of deamino-NADP has been very useful for

* This work is supported by a grant from the World Health Organization (International Reference Center for G6PD Variants) and by US Public Health Service grants GM 15253 and HE 07449 from the National Institutes of Health.

[1] Research Professor, Department of Medicine (Division of Medical Genetics), University of Washington, Seattle, Wash., USA.

[2] Chairman, Division of Medicine, City of Hope National Medical Center, Duarte, Calif., USA.

[3] Professor of Medicine and Genetics, University of Washington, Seattle, Wash., USA.



Update/Le point

Glucose-6-phosphate dehydrogenase deficiency*

WHO Working Group[1]

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the commonest enzyme disorder of human beings and a globally important cause of neonatal jaundice, which can lead to kernicterus and death or spastic cerebral palsy. It can also lead to life-threatening haemolytic crises in childhood and at later ages, by interacting with specific drugs and with fava beans in the diet. The complications of G6PD deficiency can largely be prevented by education and information, and neonatal jaundice can be successfully treated by phototherapy, a cheap and simple approach suitable for use in primary health care.

This update describes developments in the methodology for characterizing G6PD deficiency, recent knowledge of the factors that can cause haemolysis, community approaches for prevention of haemolytic crises and neonatal jaundice, and the implications of recent advances at the DNA level.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the commonest disease-producing enzyme disorder of human beings. More than 300 variants of G6PD characterized by standard methods are now known, and the recent isolation of the *Gd* gene promises important fundamental advances in the understanding of enzyme structure and function.

Since comprehensive reviews already exist (*1-3*), this article deals with recent developments in the methodology for characterizing G6PD deficiency, its epidemiology and the factors that can cause haemolysis, re-evaluation of its relevance for public health, community approaches for prevention of haemolytic crises and neonatal jaundice, and the implications of recent advances at the DNA level.

Role of the enzyme

Glucose-6-phosphate dehydrogenase is a "housekeeping" enzyme, vital for the life of every cell. Complete absence of the enzyme is unknown in the human species. Within the restricted metabolism of the red cell, G6PD occupies a particularly important place. It catalyses the first step in the hexose monophosphate pathway, converting glucose-6-phosphate to 6-phosphogluconolactone and reducing the cofactor nicotinamide-adenine dinucleotide phosphate (NADP) to NADPH. The second enzymic step in the pathway is also associated with the reduction of NADP to NADPH.

In the red cell this pathway is the only source of NADPH, which is necessary to protect the cell and its haemoglobin from oxidation in view of their role in oxygen transport. The –SH groups of several enzymes and of the β-chain of haemoglobin are particularly vulnerable to oxidation, with potentially serious consequences. Protection against oxidation is mediated by glutathione which is actively synthesized and is present in high concentration in red cells, almost entirely in the reduced form (GSH) (Fig. 1). The latter can restore oxidized –SH groups, and reacts with peroxides via glutathione peroxidase, becoming itself oxidized (to GSSG) in the process. NADPH is required for regeneration of GSH by the enzyme glutathione reductase; this is considered to be the most important function of NADPH in the red cell.

Though G6PD deficiency affects every cell in the body, its primary effects are haematological because the red cell has no alternative source of NADPH. Other more complex types of cells are protected by additional enzyme systems (such as the less specific

* This article is based on the updated version (March 1989) of the report of a WHO Working Group on Glucose-6-Phosphate Dehydrogenase Deficiency, which met in Geneva on 3-4 September 1985. Requests for single copies of the full report (document WHO/HDP/WG/G6PD/85.9) or reprints of this article should be sent to Hereditary Diseases Programme, Division of Noncommunicable Diseases, World Health Organization, 1211 Geneva 27, Switzerland. A French translation of this article will appear in a later issue of the *Bulletin*.

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Reprint No. 5020

Global **Malaria** Programme

World Health Organization

Proposed classification schemes

WHO Scientific Group 1966

- List of variants; no formal classification
- Clear phenotypic separation of: *acute haemolytic anaemia* (AHA) versus *chronic haemolytic anaemia* (CNSHA)
- (-) indicates 25% or less activity; (+/-) indicates 25-65% activity; (+) indicates normal activity (65-150%); (++) indicates greater than 150% activity

Yoshida, Beutler & Motulsky, *Bulletin of WHO*, 1971:

List of variants in 5 classes:

- Activity <10% of normal, severe enzyme deficiency with chronic non-spherocytic haemolytic anaemia (CNSHA).
- Activity <10% of normal, severe enzyme deficiency
- Activity 10-60% of normal, moderate to mild enzyme deficiency, intermittent acute hemolysis
- Very mild or no enzyme deficiency (60-100% of normal)
- Increased enzyme activity (more than twice normal)

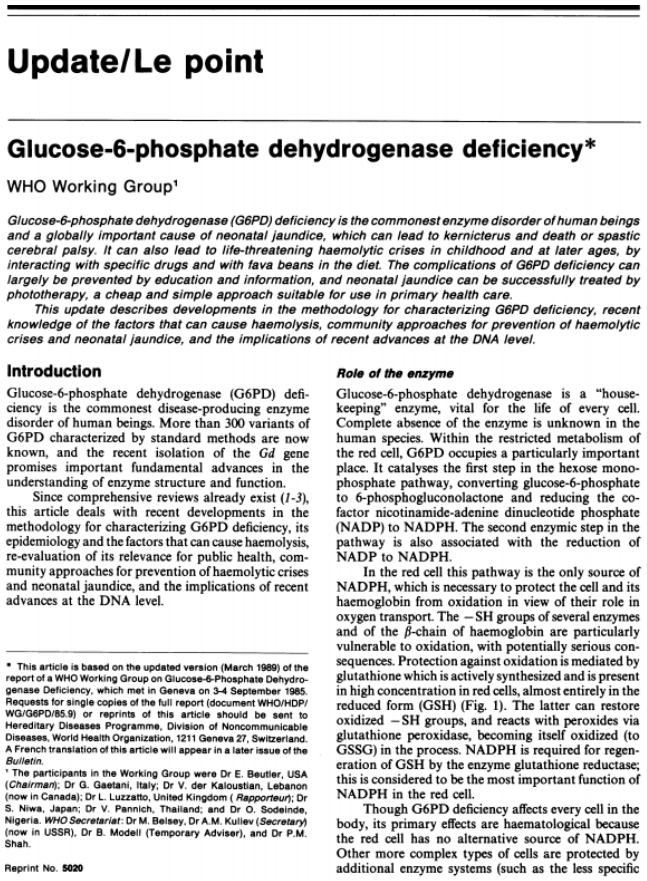
Cautionary statements: "*for purposes of convenience, the variants described in the accompanying table are somewhat arbitrarily divided into five classes*" and after "*the distinction between these classes is not always clear*"

Since 1971 this has been referred to as the 'WHO classification'

Current WHO G6PD classification

WHO working group 1985

- Class I -associated with chronic non-spherocytic haemolytic anaemia (CNSHA)
- Class II -severely deficient: less than 10% residual activity
- Class III-moderately deficient: 10-60% residual activity
- Class IV-normal activity: 60-150%
- Class V -increased activity



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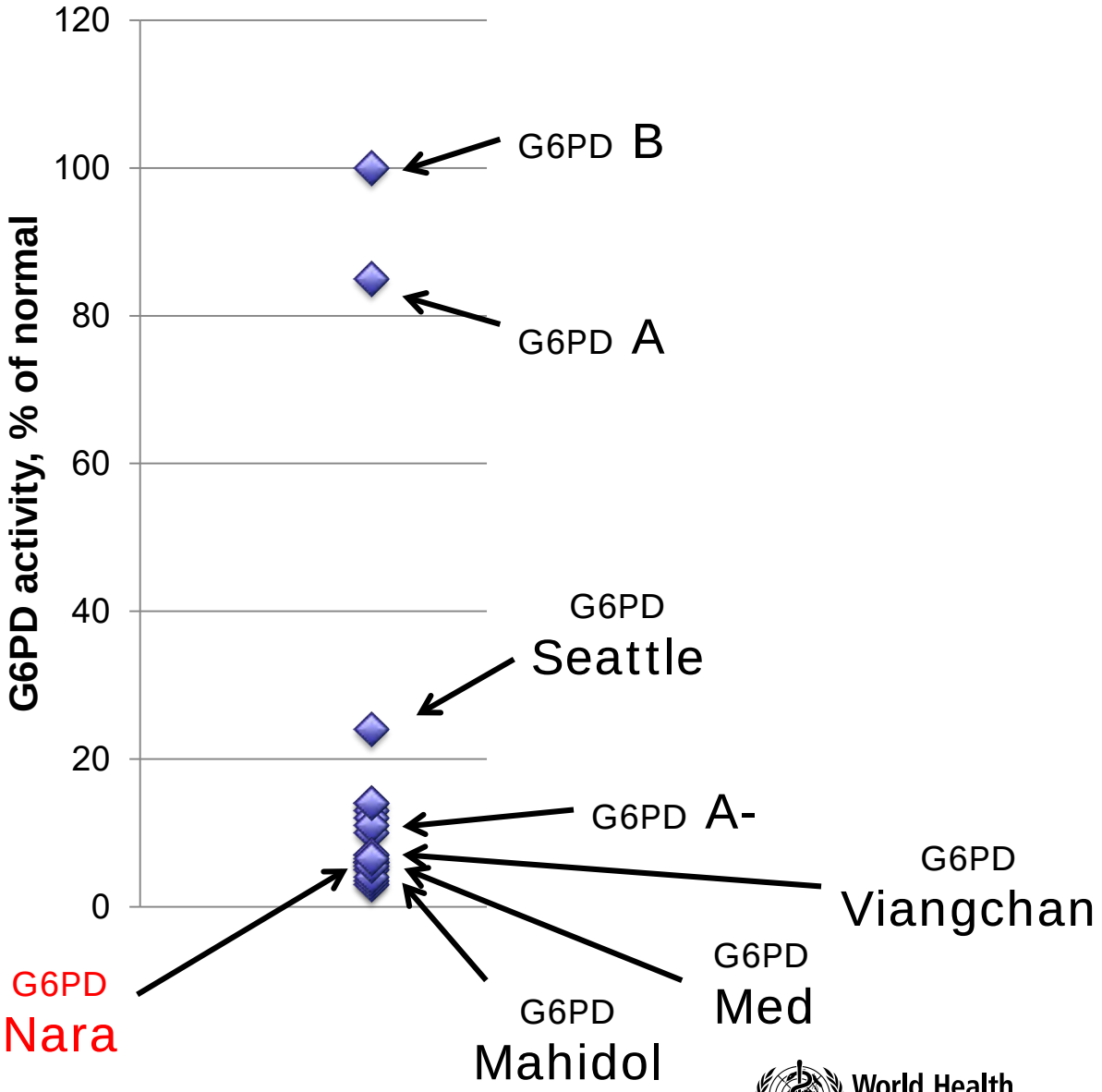
Reprint No. 5020

G6PD variants and enzyme activity

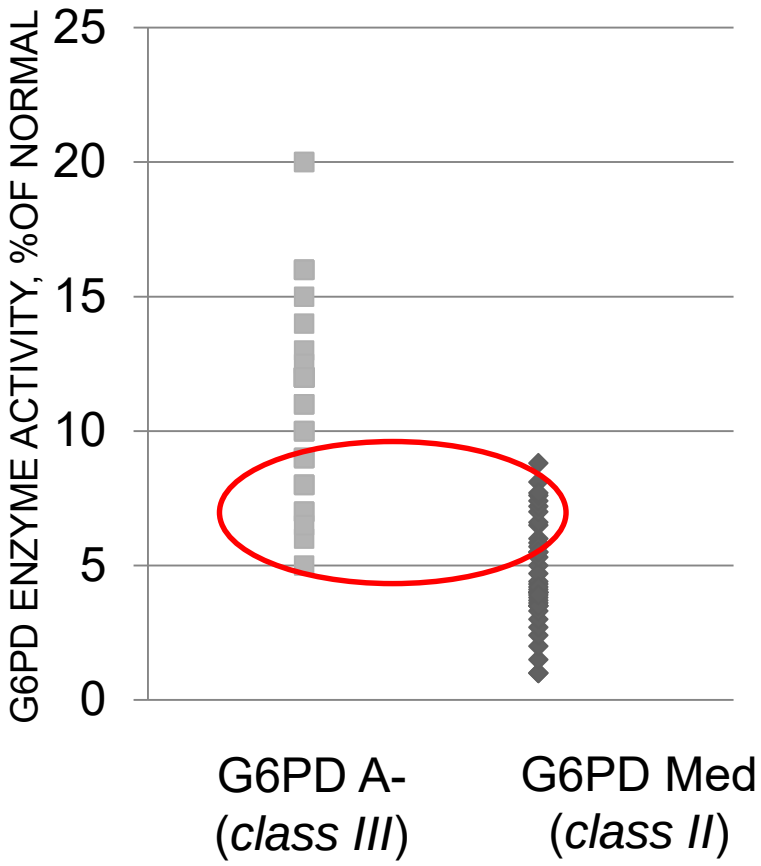
Distribution of a sample of 20 polymorphic G6PD variants in relation to mean residual enzyme activity

(courtesy of Prof L. Luzzatto)



Need for updating G6PD classification

Distributions of enzyme activity among samples male subjects with 2 different variants of G6PD deficiency

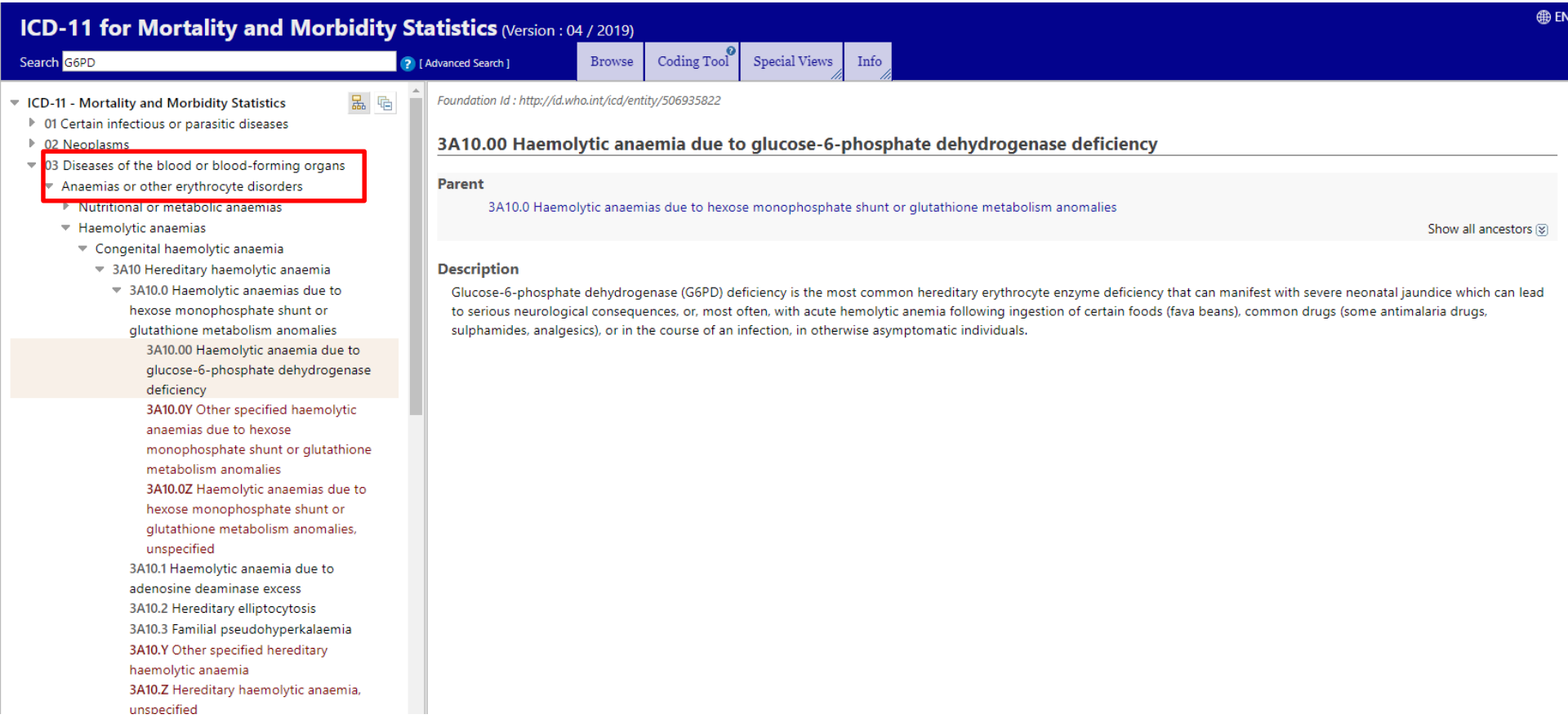


Additional reasons to update the G6PD classification:

- variable definitions of normal (> 70%, >80% residual activity)
- Only one reported case with > 150% activity
- Combine biochemical and molecular characterization

Courtesy of Prof. Lucio Luzzato

Additional reason: ongoing ICD11 revision



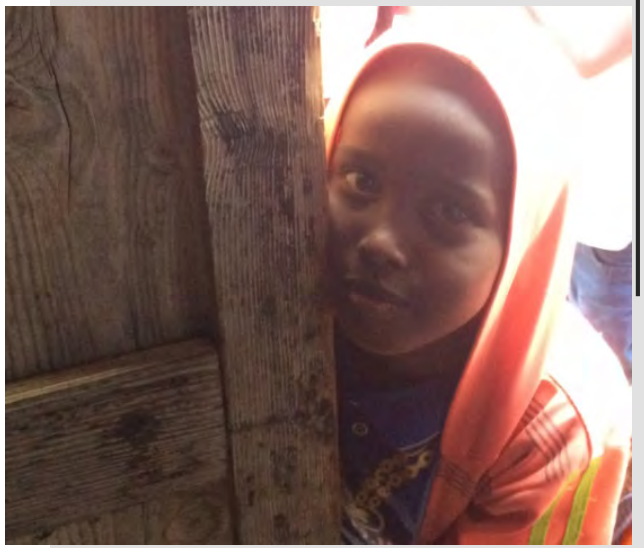
- Propose to revise based on new classification and additional clinical manifestations

International Classification of Diseases - 11

- Serendipitous finding: ICD no capture of G6PD deficiency as genetic condition, only as it is associated with clinical manifestation (AHA)

> **ICD-11: 3A10.00 – haemolytic anaemia due to G6PD deficiency**
> Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common hereditary erythrocyte enzyme deficiency that can manifest with severe neonatal jaundice which can lead to serious neurological consequences, or, most often, with acute hemolytic anemia following ingestion of certain foods (fava beans), common drugs (some antimalaria drugs, sulphamides, analgesics), or in the course of an infection, in otherwise asymptomatic individuals.

- Neonatal screening not captured
- Point of care testing options may expand
- Process for revising ICD is based on expert proposal
- Next revision February 2020



Many thanks
for your kind attention

Malaria Policy Advisory Committee Meeting
2–4 October 2019, Geneva, Switzerland
Background document for Session 8

Report of the second meeting of the Malaria Elimination Certification Panel

14–16 May 2019
World Health Organization, Geneva, Switzerland

This document was prepared as a pre-read for the meeting of the Malaria Policy Advisory Committee and is not an official document of the World Health Organization.
WHO/CDS/GMP/MPAC/2019.11

Report of the second meeting of the Malaria Elimination Certification Panel

14–16 May 2019
World Health Organization, Geneva, Switzerland

CONTENTS

ABBREVIATIONS

GMP	Global Malaria Programme
MEAT	Malaria Elimination Assessment Tool
MECP	Malaria Elimination Certification Panel
MOH	ministry of health
MPAC	Malaria Policy Advisory Committee
WHO	World Health Organization

EXECUTIVE SUMMARY

On 14–16 May 2019, the World Health Organization (WHO) convened the Malaria Elimination Certification Panel (MECP) for its second meeting to discuss potential certifications of malaria elimination to two countries, Argentina and Algeria; to review and improve the tools that have been developed to assist countries in preparing for certification; to review and improve the standard operating procedures of precertification and certification missions and to review and improve the expected certification plan for 2019–2021.

Conclusions and recommendations

- The MECP concludes that both Argentina and Algeria have met the current WHO criteria and thus recommends they should be certified malaria-free.
- The MECP agrees that the WHO criteria for elimination of the past three consecutive years with zero indigenous cases is clarified to mean the past 36 months with zero indigenous cases.
- It is essential for countries approaching elimination to investigate and document all malaria cases, including a thorough attempt to establish the location where the infection was contracted. This investigation and documentation should include all cases, whether classified as imported, introduced, indigenous etc. during the 3 years prior to expected certification.
- For countries or areas approaching elimination or working to prevent re-establishment of transmission, an adequate surveillance system should ensure that all cases that meet the suspected case definition are tested for malaria, positive cases are notified and treated, and appropriate response activities are implemented promptly. To be able to monitor the performance of the surveillance system, we recommend that registers or electronic databases for inpatient admissions and outpatient visits should include critical risk factor information that are included in the suspect case definition.
- The WHO's Malaria Elimination Assessment Tool specifies requirements for certification. Countries are advised to use this tool to monitor their progress towards elimination and assess their readiness for certification.
- A template was developed for the national elimination report required by WHO at the start of the certification process. This template was developed to ensure that critical data and information needed for a decision on certification is presented in a systematic way to the MECP. Countries are advised to use this template, although flexibility in the presentation of data is permitted.
- WHO-led precertification missions should follow a standardized format and methodology, aiming to prepare countries for a final certification mission from the MECP. The results of precertification missions should be shared with the MECP.
- The concept of regional certification means certification is applied to a group of countries in a defined geographic area or WHO region. No conclusion was reached at this meeting with respect to processes for regional certification, and further discussions will be held at future meetings.
- Countries with a plan to request WHO certification of malaria elimination are strongly encouraged to discuss with WHO to begin preparations well in advance so that a certification process could be successfully completed and will take into account the complexity of epidemiological situation in each country.

BACKGROUND

The Malaria Elimination Certification Panel (MECP), established by WHO in 2017, is charged with recommending to the WHO Director-General, through the WHO Malaria Policy Advisory Committee and the Global Malaria Programme (GMP), whether malaria elimination can be certified in applicant countries based on WHO criteria. During the first MECP meeting held from 13–14 December 2017, the members reviewed and made recommendations for improvements to two draft guides on certification: one for countries applying for certification, and one for the MECP panel conducting the certification evaluation. It was suggested that the guides be piloted during the certification processes for the first two countries to be certified, and be reviewed again by the MECP before their publishing.

After the establishment of the MECP, two countries, Paraguay and Uzbekistan, were certified in 2018. Additionally, certification evaluation missions were completed in two applicant countries, Argentina and Algeria, in March and April 2019, respectively. More and more countries are making progress towards elimination and achieving important milestones. The need to support and prepare these countries for certification is increasing.

During the three-day meeting, 11 members, two ad hoc MECP members and the WHO Secretariat discussed the issues raised from recent certifications, the potential certifications of malaria elimination in Argentina and Algeria, and the tools that have been developed to assist countries to prepare for certification and to assist the MECP and WHO in the certification evaluation. (See Annex 1 for the meeting agenda and Annex 2 for a list of participants.)

DECLARATION OF INTERESTS

All MECP and ad hoc members participating in the meeting submitted a declaration of interests that was assessed by the Elimination Unit, GMP at WHO. Based on the assessment, Dr Keith Carter was partially recused from the discussion of certification to Argentina and was not part of the decision-making in the final recommendation. Professor Daouda Ndiaye was partially recused from the discussion of certification to Algeria and was not part of the decision-making in the final recommendation on certification to Algeria.

OBJECTIVES

The purpose of the second annual MECP meeting was to discuss the potential certification of malaria elimination in Argentina and Algeria, and to review and improve the tools for WHO certification of malaria elimination.

Specific objectives

(1) Discuss the potential certifications of malaria elimination in Argentina and Algeria.

(2) Improve the tools for WHO certification of malaria elimination:

- Malaria Elimination Assessment Tool (MEAT), including requirements for certification;
- national elimination report template; and
- standard operating procedures for WHO precertification missions and final certification evaluation missions, and template of mission report.

(3) Review and comment on the proposed certification work plan for 2019–2020:

- draft work plan for potential certification in 2020 -2021;
- regional certification of malaria elimination in the WHO European Region.

PROCESS AND PRESENTATIONS

Background documents

In preparation for the meeting, Dr Allan Schapira and Professor Mahamadou A. Thera prepared a report based on their findings from the certification evaluation mission to Algeria from 25 March to 5 April 2019. After the certification evaluation mission, Algeria conducted a series of activities to improve its system based on recommendations made by the two team members. Algeria submitted additional documents that described all activities conducted after the certification mission. The two team members, after reviewing those documents, prepared a report to supplement their certification mission report.

Professor Rossitza Mintcheva and Dr Martha L. Quiñones prepared a report based on their findings and conclusions from the certification evaluation mission to Argentina on 18 March–29 March 2019.

The WHO Secretariat prepared the MEAT with specified requirements for certification, a template for the national elimination report, the standard operating procedures for WHO precertification missions and MECP certification evaluation missions, and a certification mission report template.

Meeting opening

The second MECP meeting opened with a welcome from Dr Pascal Ringwald, coordinator of WHO GMP, on behalf of Dr Pedro Alonso.

Dr Ringwald thanked the members of the MECP for their commitment and contribution to this important work. Certification of malaria elimination is a mandate given to WHO by Member States that officially recognizes a significant public health achievement made by countries and generates momentum to the global community in the fight against malaria.

Globally, progress on malaria has stalled in recent years, Ringwald said, the world is not on track to reach its 2030 targets regarding reduction of morbidity and mortality in the Global Technical Strategy. Meanwhile, more countries are progressing towards elimination.

To continue recognizing and celebrating the important achievement of elimination is not only important to the countries that have achieved elimination but doing so provides inspiration and motivation to other countries and sets an example for countries that are endemic for malaria.

Dr Kim Lindblade joined Dr Ringwald in welcoming the MECP members and thanked them for their efforts in the WHO certification process. She said that as more countries move toward the goal of elimination in the next few years, requests for certification will increase.

Session 1: Setting the scene

Dr Li Xiao Hong reviewed the meeting agenda, meeting objectives and expected outcome. No objections were made to the proposed agenda.

During his presentation, Dr Jose Najera shared his thoughts on the certification of malaria elimination. He reviewed malaria surveillance in several countries, including Sri Lanka, Paraguay, Uzbekistan, Argentina and others; the use of indicators of the annual blood examination rate and the slide positive rate; and the combined use of active and passive case detection in different countries. That these countries are certified malaria-free and have remained so indicates that malaria surveillance in these countries is adequate.

Nevertheless, Dr Najera said it appeared that the interpretation of the adequacy of surveillance among these countries varied significantly, perhaps due to differences in their social and ecological backgrounds. In general, there is room to improve surveillance, even in countries that are close to or have achieved elimination. Surveillance could be enhanced when the search for cases is guided by a clear understanding of where, when and why malaria cases might occur, rather than by simply testing more blood samples. Surveillance could also be improved when epidemiological analysis is conducted at the periphery level and attention is paid to any cases clustered in time and space.

The definition of "suspected malaria" is a dynamic concept that may change over time as transmission is reduced. Cases might be missed if fever is used as the only indicator. To assess the risk of re-establishment of malaria transmission, factors that should be considered include historical foci where transmission had been active and approaches that had been used to clear these foci; whether ecological changes, including land use, favour vector proliferation or the opposite; and the pattern of migrant movement. To prevent re-establishment of endemicity, response to imported cases is critical.

Dr Lindblade reviewed the two criteria for certification of malaria elimination and emphasized that the assessment of adequacy of the surveillance system is critical to both criteria. A number of attributes should be considered when assessing a surveillance system, but some, such as data quality, sensitivity and timeliness, are more important for elimination. Ideally, a passive surveillance system should detect 100% of cases that have occurred. But when several elements are taken into account – including the probability that clinical symptoms are presented, the probability that patients will seek health care, the probability that a suspected case is tested, the sensitivity of a diagnostic test, and the probability of reporting positive cases – the sensitivity will not reach 100%. In the absence of cases, methods that are used to assess sensitivity cannot be applied. Assessment of the sensitivity of surveillance should focus on whether all suspected cases are tested for malaria, and whether all positive cases are reported.

Dr Lindblade also presented the GMP's concerns about the use of the annual blood examination rate (ABER) in elimination settings as an indicator of vigilance. ABER is defined as the number of people receiving a parasitological test for malaria per unit population per year. The denominator of the ABER, i.e. the population at risk, is difficult to define when transmission is very low. She noted that what should be measured is the probability that a surveillance system would detect a case should it occur. She suggested that the freedom from infection (FFI) statistical methods, which were developed to quantify the likelihood that disease would be detected if it existed, might be useful to guide countries on where surveillance improvements are required. Issues on introduced cases and others were presented for the panel to consider and discuss.

Conclusions from the two presentations:

1. Measurement of sensitivity and adequacy of surveillance

   → ABER as an indicator to assess the adequacy of surveillance

ABER was an indicator first introduced in the Global Malaria Eradication Programme when routine passive surveillance systems were considered inadequate. The use of ABER in elimination was

proposed by the Expert Committee of malaria in its 8th report[1], as requested by national programmes, who needed field applicable criteria to prove an adequate search for malaria cases. The expert committee proposed an ABER of at least 10% of the population covered by surveillance as a minimal level of testing for a surveillance system, but 10% ABER threshold could not be justified from an epidemiological perspective. The denominator of ABER, population at risk, is difficult to calculate in elimination settings. ABER doesn't provide information on whether the blood samples tested for malaria are truly from the population of concern. High rates of blood examination can be achieved through active case detection of easily accessible populations, which is not the purpose of blood sample testing. When transmission becomes focal, it could be highly inefficient to maintain a high blood examination rate. In this regard, countries are advised not to use ABER as a key indicator of adequate surveillance but to focus on ensuring that all suspected cases are tested, reported and responded to.

→ Slide positivity rate (SPR) as an indicator of the adequacy of surveillance

The SPR, defined as the proportion of blood smears found to be positive for Plasmodium among all blood smears examined, could differ significantly between active and passive surveillance. Seasonality, the population targeted, health care-seeking behaviour and suspected case definition will also change the SPR. The use of only the SPR to understand the specificity of surveillance is inadequate.

Conclusion: There are yet no fully satisfactory methods or metrics for measuring surveillance sensitivity in elimination settings. Countries are advised to focus on whether all suspected cases are tested for malaria, and whether all positive cases are reported. Practically, surveillance can be improved when blood sample testing is guided by a clear understanding on where, when and why malaria cases might occur, rather than by simply testing more blood samples.

2. Introduced cases

An introduced case is contracted locally, with strong epidemiological evidence linking it directly to a known imported case (first-generation local transmission).

- → There is limited practical value in classifying cases as introduced in areas of known transmission.
- → When countries are very close to elimination and are about to meet the criteria for certification, identifying introduced cases is important. Case classification should be reviewed and rigorously verified to ensure the evidence provided for case classification is adequate.
- → In principle, there should be an epidemiological link between an introduced case and a known imported case.
- → Malaria programmes should make all possible efforts to trace the source of infection of introduced cases.

3. WHO has stated that countries are eligible to request malaria-free certification from WHO when they have reached zero indigenous malaria cases for at least the past three consecutive years. Recognizing that the last indigenous case might occur in any given month of the year and that it takes time to complete a certification process, the MECP has clarified that three years is equivalent to 36 months, rather than three calendar years.

4. The MECP and WHO should follow up with countries that are certified malaria-free to validate the usefulness of the recommendations made by the MECP, to inform future practices in the prevention of re-establishment of transmission.

1 Expert Committee on Malaria, eighth report. www.who.int/iris/handle/10665/40477

Review the terms of reference of MECP and the certification process

The MECP is entrusted with making a recommendation to WHO on whether an applicant country should be certified malaria-free based on current criteria and with providing technical advice on the criteria and procedures for certification, when needed. The MECP also recommends to WHO whether decertification is warranted in a certified country should the country meet the minimum criterion of re-establishment of transmission. The use of different documents generated in a certification process was clarified. At the end of a certification evaluation mission, mission members will provide a briefing to the Ministry of Health (MOH) that includes their conclusions about the mission and their recommendations. The conclusions and recommendations made by mission members during their briefing to MOH only represent the views of the certification mission members. They do not represent the views of the MECP or WHO. The certification evaluation report, developed by mission members, is to provide details on the observations and findings of the certification mission to all members in the MECP and WHO Secretariat. The broader use of this evaluation report will be further explored by the GMP in consultation with WHO regional office and others.

Recommendation

The MECP recommends that the WHO Secretariat further detail the process of decertifying countries, including the process for collecting annual reports on malaria cases from certified countries, assessing the quality of the surveillance data, investigating reported cases and evaluating whether the country has met the criteria for re-establishment of transmission.

Session 2: Certification of malaria elimination in Argentina

Dr Mintcheva, team leader of the Argentina certification mission (18–29 March 2019), briefed the MECP on the activities conducted by the evaluation team, their observations and findings. The team made visits to key institutions and organizations in the autonomous city of Buenos Aires (CABA) and the provinces of Buenos Aires, Salta and Jujuy. The certification mission concluded that Argentina has met the two criteria of WHO certification and recommended that Argentina be certified malaria-free.

Following the briefing, other MECP members raised concerns or queries on several issues, which were clarified and addressed by the mission members and the WHO Secretariat. The concerns and queries included: cross-border collaboration; case classification methods and verification; microscopy proficiency testing and sustainability of quality control for malaria diagnosis; the rationale and implementation of reactive vector control; surveillance in the armed forces and police; and health system integration and the quality of primary health care. The panel noted that cross-border collaboration between Argentina and Bolivia was not limited to information-sharing but included active participation of Argentinian MOH staff in conducting indoor residual spraying and active case detection in collaboration with the Bolivian health team on the Bolivian side of the border. The cross-border collaboration has been successful and sustainable; such experiences should be shared with countries experiencing border malaria issues. Argentina has undertaken an extensive integration effort, moving from a vertical program overseen by the national MOH to an integrated programme within the provincial health and surveillance systems.

After the discussion, the MECP agreed with the conclusions of the certification mission members – that mosquito-borne local malaria transmission in Argentina has been interrupted throughout the country and that the existing health system should be able to prevent re-establishment of transmission.

The MECP unanimously recommended that Argentina should be certified as malaria-free.

Session 3: Certification of malaria elimination in Algeria

Dr Schapira, team leader of the Algeria certification mission (25 March – 5 April 2019), briefed the MECP on the activities conducted by members of the certification mission team, their observations and findings. The team reviewed a number of supporting documents, including national elimination report before their visit to the country. They visited the MOH and the two national institutes involved in malaria elimination: the National Institute of Public Health (INSP) and the Institut Pasteur of Algeria (IPA). Three wilayas (provinces) were selected for field visits: Ouargla, Adrar and Tamanrasset.

At the end of the certification mission, the team concluded that local malaria transmission had been interrupted in Algeria for at least the past three consecutive years. The certification mission identified some weaknesses of the health system and recommended that the MOH of Algeria make immediate improvements of the vigilance of health care providers in relation to suspecting malaria and the capacity and quality of case investigation in the vulnerable wilayas. The MOH of Algeria submitted a supplementary report to the mission members (copied to WHO secretariat) by email on 8 May. This report detailed the actions taken by the Algerian MOH in line with the recommendations of the mission.

Dr Schapira and Dr Thera, after reviewing the supplementary report concluded that the immediate, vigorous and technically appropriate actions taken by the MOH meant that the health system would from then on be able to prevent re-establishment of transmission.

Following the briefing, the MECP raised concerns about: case classification, such as whether reported introduced and imported cases were correctly classified; programme management; and the sustainability of malaria-free status in a large country with major cross-border population movements; the quality of vector control operation; the correctness of suspected case definition; the use of Rapid Diagnosis Tests; structures at the subnational level that are responsible for active case detection; for all of these, the situation and the developments in Algeria were clarified by the team members and the WHO Secretariat.

The panel concluded that Algeria has met the certification criteria in spite of facing a very challenging situation, due to its long, porous borders with neighbouring countries that are endemic for malaria and the presence of competent vectors.

The MECP unanimously recommended that Algeria should be certified malaria-free. The MECP recommended that the WHO Secretariat should follow up closely with the national malaria program in Algeria to ensure the weaknesses identified during the certification mission are rectified. A mission team from the WHO Secretariat could be sent to the country to confirm the implementation of the recommendations made by the panel.

Session 4: Recommendations on the tools developed for certification

Malaria elimination assessment tool with specified requirements for certification

The MEAT was developed to assist countries to operationalize the WHO *Framework for malaria elimination*, including the certification criteria. This tool is developed to assist malaria-eliminating countries in monitoring achievement of elimination milestones and assess the readiness for certification. This tool, together with the template of the national elimination report, will be included in the guide for countries preparing for WHO certification of malaria elimination.

The overall comments from MECP members on this tool were positive. Members recognized the practical needs of malaria-eliminating countries in preparing for certification and appreciated the efforts that have been made to develop this tool with specific requirements for certification of malaria elimination. The MECP said the critical requirements for certification are well-captured in the tool and the stringency of different elements required for certification are reasonably differentiated. However, the panel advised that the requirements for certification specified in this tool should be used as a guide by countries and do not indicate decisive rules for certification. Flexibility may be allowed when the requirements are applied to different countries, recognizing that certification of malaria elimination is a complex exercise.

Template for the national elimination report

The national elimination report is a key document that applicant countries submit to WHO and the MECP for review to consider whether certification should be granted. It provides evidence that malaria transmission has been interrupted, resulting in zero indigenous cases in at least the past three consecutive years, and that the surveillance and response system will be able to prevent re-establishment of transmission. The template was developed to help countries develop their national elimination report.

Major recommendations for improvement on this template:

1. While the template should ensure that essential and critical data and information are included in the report, it should allow some freedom for countries to describe what they believe is important for certification of malaria elimination.

2. How malaria elimination was achieved in the country and how elimination is going to be sustained in the future through prevention of re-establishment are equally important for certification.

3. Health indicators should be revised to be in line with recommended indicators that are used to assess the quality of health service by WHO.

4. General, geographical and ecological information should be specifically related to malaria.

5. The number of tables should be reduced and should focus more on data from recent years.

6. For prevention of re-establishment of transmission, countries should describe what has been done in the last three years to maintain zero indigenous cases, what will be changed in the future, whether and how these activities or strategies will be sustained in the future.

7. Data or documents needed for certification, as specified in the MEAT, should be linked to the national elimination report.

Standard operating procedures for WHO precertification visits and missions of the MECP, and template for the certification evaluation mission report

Standard operating procedures (SOPs) were developed to provide practical guidance to WHO staff and MECP members who visit a country to assess the preparedness for certification of malaria elimination. It describes the objectives of WHO precertification missions and MECP certification evaluation missions, and clarifies the roles and responsibilities of participants in a mission. It provides guidance on defining the agenda of a certification evaluation mission to ensure that the objectives of the mission can be achieved in a set timeframe. It provides principles for site selection and suggests methodology and tools to be used in assessing different technical areas, including the competency of staff at the national and subnational levels. The template for the certification evaluation mission report was developed to help team members develop their mission report quickly and efficiently

while ensuring that the two major questions for certification are adequately answered. This SOP is for internal use of WHO staff and MECP members.

The MECP concluded that this tool is useful and will be beneficial for future certification evaluation missions.

A regional approach to certify malaria elimination in the WHO European Region

The *Global technical strategy for malaria 2016–2030* (GTS), adopted by the World Health Assembly in May 2015, reiterates the ultimate vision of achieving a malaria-free world. In line with the global vision, the WHO European Region, after reaching zero indigenous malaria cases in 2015, has committed to prevent re-establishment of transmission in the region.

WHO's mandate to certify countries malaria-free status comes from a resolution endorsed by the 13th World Health Assembly in 1960, which "requests the Director-General to establish an official register listing areas where malaria eradication has been achieved, after inspection and certification by a WHO evaluation team". The first list of official register was published in 1963. Because the official register was restricted to countries that achieved elimination with specific measures, a list was created to list countries "where malaria never existed or disappeared without specific measures", in order to supplement the official register. The first supplementary list was first published in 1963. The supplementary list was latest updated in 2012, adding 52 more countries. Some countries, listed in the supplementary list, such as Maldives and Malta, had requested for certification. The European region currently has 20 countries listing in the official register, 30 countries in the supplementary list, and 4 - neither certified nor listed in the supplementary list. Taken all these together, a regional approach to certify the whole European region as malaria-free was first proposed during the inaugural meeting of malaria elimination certification panel (MECP).

Following on the discussion from the first MECP meeting, the WHO Regional Office for Europe, in collaboration with GMP, convened a technical consultation in Baku, Republic of Azerbaijan, from 26–27 November 2018. The technical consultation concluded that all countries and territories in the WHO European Region have reported zero indigenous cases for at least the past three consecutive years and the surveillance and response system in place appeared to be adequate to prevent re-establishment of transmission. Regional certification of malaria elimination was considered feasible and could be recommended for the WHO European Region. The Baku meeting proposed criteria and drafted methodology for regional certification.

The MECP noted that a regional approach to certify WHO EURO malaria is in line with the vision of a malaria-free world and regional malaria elimination goals. The MECP recognized that the regional approach may be an efficient way to certify multiple countries that had been malaria-free for a long period of time and that there might be additional value using a regional approach to conduct certification, especially when a region or sub-region is geographically related and shares a similar ecological environment. However, while the MECP is not against a regional approach for certification, it believes that higher priority should be given to certifications requested by individual countries in the short term, given that the number of countries requesting certification is on the rise.

Review workplan for 2020-2021

Dr Li Xiao Hong presented a proposed plan of certification in the next two years. The MECP suggested that countries with a plan to request WHO certification of malaria elimination should be strongly encouraged to reach out to the WHO secretariat to begin preparations well in advance so that a certification process could be successfully completed and will take into account the complexity of epidemiological situation in each country.

ANNEX 1. AGENDA

Chair: Brian Greenwood

Day 1 – Tuesday, 14 May 2019		
Session 1 Setting the scene		Presenter/facilitator
8:30 – 9:00	Registration	Secretariat
9:00 – 9:15	Welcome and opening of meeting Introductions Group photo	Pascal Ringwald
9:15 – 9:30	Declaration of interests Meeting purpose and objectives	Li Xiao Hong
9:30 – 10:10	WHO certification of malaria elimination: some thoughts from the Global Malaria Eradication Programme and recent certification practices (20') Discussion (20')	José Nájera
10:40 – 11:30	Discussion of indicators and issues related to certification	Kim Lindblade
11:30 – 12:30	Review the terms of reference of MECP members and the operating procedures for certification Discussion	Li Xiao Hong
Session 2 Discussion of certification of malaria elimination in Argentina		
13:30 – 14:00	Briefing from the certification mission	Rossitza Mintcheva
14:00 – 15:30	Q & A	
16:00 – 17:00	Conclusions and recommendations	Brian Greenwood
17:00 – 17:15	Summary of Day 1	

Day 2 – Wednesday, 15 May 2019		
Session 2 Discussion of certification of malaria elimination in Algeria		
9:00 – 9:30	Briefing from the certification mission	Allan Schapira
9:30 – 10:30	Q & A	
11:00 – 12:00	Conclusions and recommendations	Brian Greenwood
Session 3 Discussion on requirements for WHO certification		
13:00 – 13:15	Introduction to the Malaria Elimination Audit Tool and requirements for certification	Li Xiao Hong
13:15 – 15:00	Discussion on requirements for certification (1) • National strategy, coordination, policies and advocacy (Fred Binka) • Surveillance (Anatoly Kondrashin) • Diagnosis (Daouda Ndiaye	Kim Lindblade
15:30 – 17:00	Discussion on requirements for certification (2) • Case management (Brian Greenwood) • Entomological surveillance and vector control (Martha Quiñones) • Prevention of re-establishment (Allan Schapira)	Kim Lindblade
Day 3 – Thursday, 16 May 2019		
Session 4 Discussion on operating procedures for certification		
9:00 – 11:00	Template for national elimination reports	Rossitza Mintcheva
	Template for certification mission report	Li Xiao Hong
Session 5 Discussion on the upcoming workplan for certification		
11:20 – 12:00	Regional certification of malaria elimination in the WHO EURO region	Li Xiao Hong
12:00 – 12:50	Work plan for 2020 - 2021	Li Xiao Hong
12:50 – 13:00	Meeting closure	Pedro Alonso

ANNEX 2. LIST OF PARTICIPANTS

Members of the Malaria Elimination Certification Panel

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GHANA

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Medical Officer
BURKINA FASO

Update on the E-2020, certification and STOP-Malaria

Kim Lindblade, Team Lead

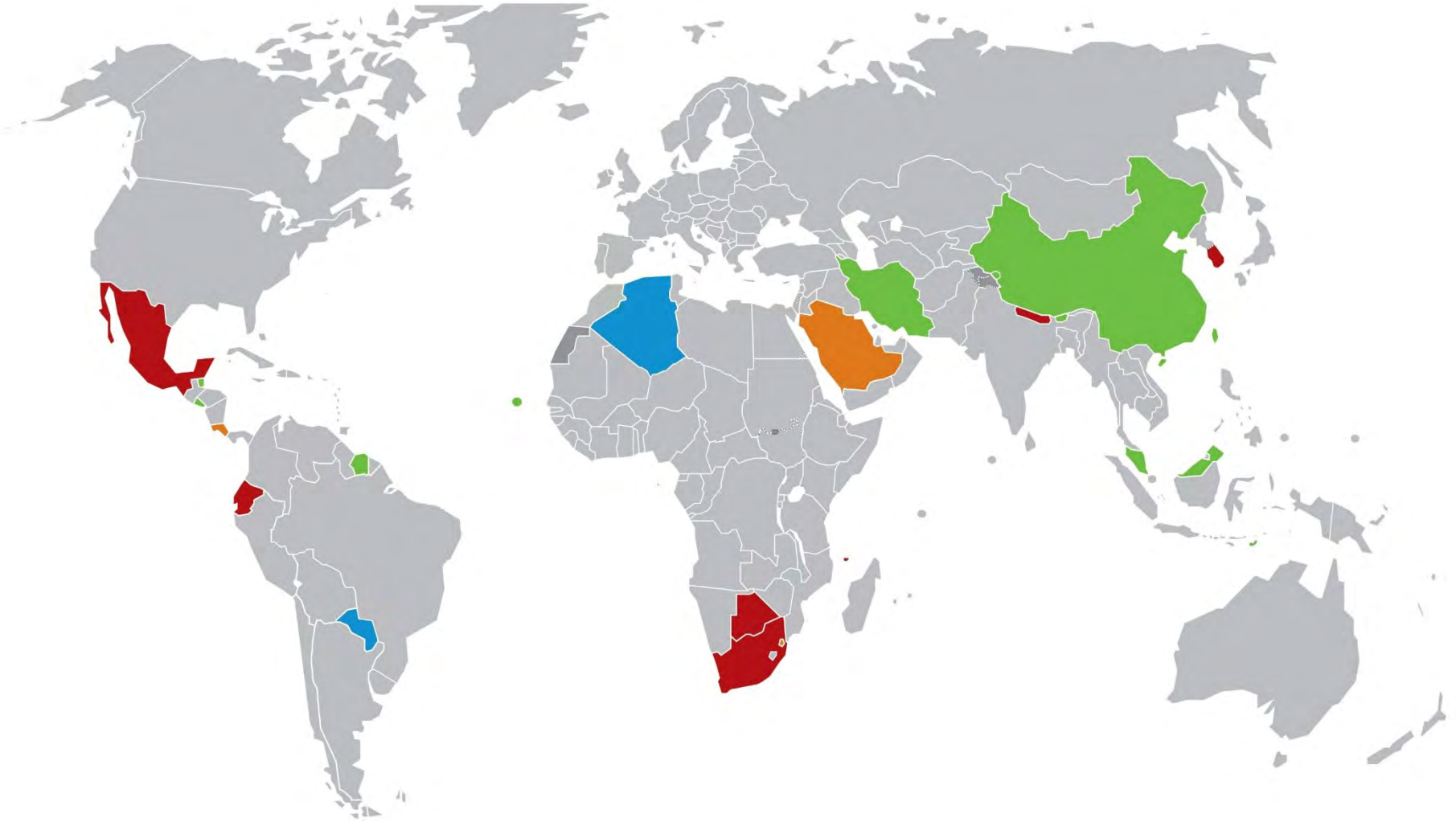
Malaria Elimination Unit

Global Malaria Programme

Global **Malaria** Programme

World Health Organization

E-2020 countries



* Preliminary figures for 2018 (Source: national malaria control programme reports); final figures will be published in the *World malaria report 2019*.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of WHO concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

2019 Global Forum of Malaria-Eliminating Countries

https://www.who.int/malaria/publications/atoz/e-2020-progress-report-2019/en/

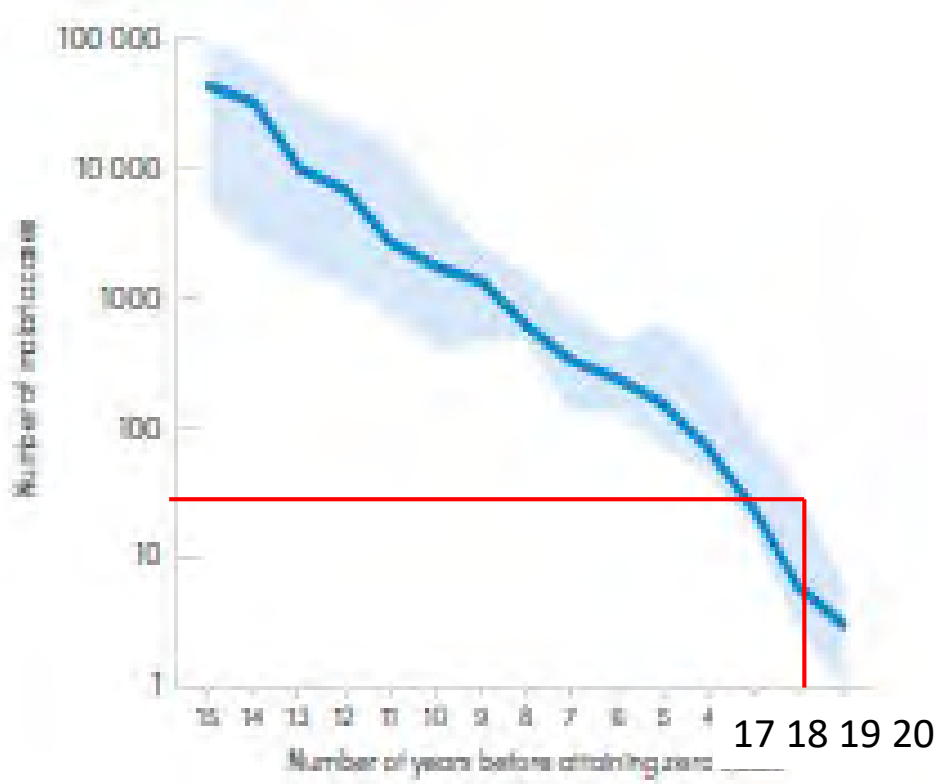
Global Malaria Programme

World Health Organization

Progress towards elimination

Median number of indigenous malaria cases in the **years before attaining zero indigenous cases** for the 14 countries that eliminated malaria between 2000 and 2015.

Red line indicates that **75% of countries reported 51 or fewer** cases two years before reaching 0.



- Certified malaria-free
- On track (less than 51 indigenous cases)
- Somewhat off track (between 51 and 166 cases)
- Off track (more than 166 cases)

Challenges – African region

Country	Challenges
Algeria	• Maintaining vigilance • Cross-border collaboration
Botswana	• Quality of case investigations • Poor uptake of vector control
Cabo Verde	• Identifying and responding to imported cases • Maintain quality of vector control
Comoros	• Resurgence to levels approaching the period before mass drug administration • Low utilization of insecticide-treated bednets
Eswatini	• Update stratification map • Improve healthcare seeking
South Africa	• Programme implementation at provincial level • Staff recruitment moratorium

Challenges – American region

Country	Challenges
Belize	• Maintaining surveillance in malaria-free areas • Classifying cases
Costa Rica	• Illegal gold mining activity • Extending case management and surveillance to undocumented and migrant populations • Lack of entomologic expertise
Ecuador	• Illegal activities (drug trafficking and mining) • Limited health system coverage in foci • Significant mobility across borders with Colombia and Peru
El Salvador	• Completing certification process
Mexico	• Integration of program into health system • Implementing use of RDTs
Paraguay	• Successful integration of malaria programme
Suriname	• Case classification • Brazilian miners from French Guiana

Challenges – Eastern Mediterranean region

Country	Challenges
Iran, Islamic Republic of	• Floods in formerly malaria-endemic areas • Competing public health priorities
Saudi Arabia	• Civil unrest in Yemen • Shortage of qualified and experienced staff

Challenges – South-East Asia region

Country	Challenges
Bhutan	• Improving quality of case investigation • Maintaining vigilance • Targeting appropriate interventions to areas with high malariogenic potential
Nepal	• Lack of malaria focal points at subnational level • Cases identified in formerly non-endemic areas that are very difficult to access • Seasonal, cross-border migration
Timor-Leste	• Preparing for certification • Lack of domestic funding for most NMCP positions • Border collaboration with Indonesia

Challenges – Western Pacific region

Country	Challenges
China	• Completing subnational verifications • Early diagnosis and treatment for imported cases (mostly nationals) • Maintaining vigilance
Malaysia	• *P. knowlesi* • Prompt diagnosis and treatment in remote areas • Undocumented migrant workers
Republic of Korea	• Vector control along the demilitarized zone • Implementation of rapid diagnostic tests • Malaria in the military • Cross-border and collaboration with Ministry of National Defense

Status of E-2020 countries as of 2018

Country	2010	2011	2012	2013	2014	2015	2016	2017	2018 [b]	↑ ↔ ↓ [c]	2020 [d]
Africa											
Algeria	1	1	55	8	0	0	0	0	0	↔	
Botswana *	1 046	432	193	456	1 346	326	716	1 900	533	↓	
Cabo Verde	47	7	1	22	26	7	48	423	2	↓	
Comoros	36 538	24 856	49 840	53 156	2 203	1 300	1 066	2 274	19 682	↑	
Eswatini	268	549	562	962	711	157	350	724	59	↓	
South Africa	8 060	9 866	5 629	8 645	11 705	555	4 323	22 061	9 562	↓	
Americas											
Belize	150	72	33	20	19	9	4	7	3	↓	
Costa Rica	110	10	6	0	0	0	4	12	70	↑	
Ecuador	1 888	1 219	544	368	242	618	1 191	1 275	1 653	↑	
El Salvador	19	9	13	6	6	2	12	0	0	↔	
Mexico	1 226	1 124	833	495	656	517	551	736	803	↑	
Paraguay	18	1	0	0	0	0	0	0	0	↔	
Suriname	1 712	771	356	729	401	81	76	40	30	↓	
Eastern Mediterranean											
Iran (Islamic Republic of)	1 847	1 632	756	479	358	167	81	57	0	↓	
Saudi Arabia	29	69	82	34	30	83	272	177	61	↓	
South-East Asia											
Bhutan	436	194	82	15	19	34	15	11	6	↓	
Nepal *	3 894	3 414	2 092	1 974	832	591	507	623	559	↓	
Timor-Leste *	48 137	19 739	5 211	1 025	342	80	94	16	0	↓	
Western Pacific											
China	4 990	3 367	244	86	56	39	3	0	0	↔	
Malaysia	5 194	3 954	3 662	2 921	3 147	242	266	85	0	↓	
Republic of Korea	1 267	505	394	383	557	627	602	436	501	↑	

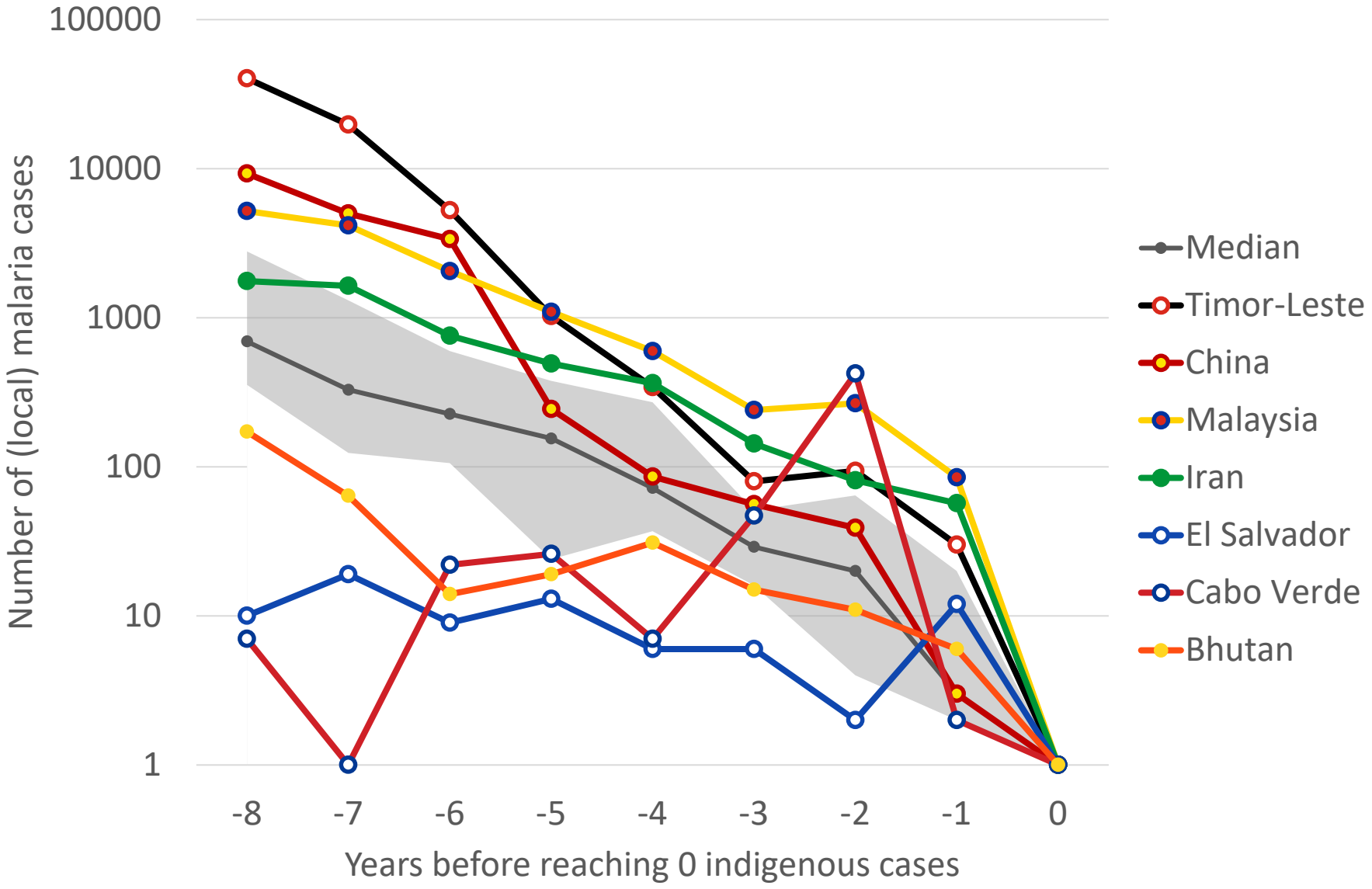
Likely to reach 0 by 2020:

Algeria*
Cabo Verde*
Belize
El Salvador*
Suriname
Iran*
Bhutan
Timor-Leste*
China*
Malaysia*
(Sri Lanka*)

*Already reached 0

World Health Organization

Rates of decrease



Changes in trajectories towards elimination

Statistics at 5 years before 0	Countries eliminating by 2015	Countries eliminating after 2015
Median no. cases	117	244
75th percentile	291	759
Median annual rate of decline	-0.37	-0.38
75th percentile	-0.42	-0.58

Rates of decline are higher 10 to 5 years before elimination (not shown)

Countries eliminating more recently have similar rates of decline but several have started from a higher number of cases

Key recommendations of the Malaria Elimination Oversight Committee at the Global Forum

- Need greater emphasis on identifying and characterizing “key populations” for malaria
- Diagnosis and treatment of malaria must be assured free of charge and without barriers to undocumented and uninsured people
    - Consider temporary policies on an emergency basis if there are significant legal barriers
- Community health workers must be able to diagnose AND treat malaria where community health workers are implemented
- WHO to develop an elimination dashboard to include key national programmatic indicators

Reflections on the E-2020 initiative

Positive aspects

- Dissemination of learning between countries and across regions
  - Changes in elimination strategies
  - Improved classification of cases
  - Shared sense of the possible
- Friendly competition and the lure of certification as motivating factors
- Focused review in conjunction with the Malaria Elimination Oversight Committee and Global Fund

Areas for improvement

- Need to clarify objectives, expectations and added value to countries of the E-2020 initiative
- More direct support to the national elimination advisory committees
- Programme audits needed more frequently
- Interaction should be elevated above programme managers

Selection of the E-2025 countries

- Global Forum to be held next Q1 2021
- Launching the new cohort in Q4 2020 or Q1 2021
    - Including E-2020 countries that have not yet eliminated
- Criteria for new countries
    - Epidemiologic threshold – based on evidence from previous countries + optimism
    - National elimination goals
    - Political commitment?
    - Health system indicators?
- Greater emphasis to be placed on country ownership of the E-2025 initiative

Certification

Recent certifications

Guidance documents

- Preparing for WHO certification of malaria elimination -- *an operational manual*
  - Target audience: NMCPs, certification committees
  - To be sent to MPAC for information and input before publication
- Standard operating procedures for WHO pre-certification and certification missions (internal)
  - Target audience: MECP members, WHO staff

Timeline for possible certifications

Region	2020	2021?
AFRO		Cabo Verde
SEARO		Timor-Leste
WPRO	China	Malaysia
PAHO	El Salvador	
EURO	Azerbaijan	Tajikistan
EMRO		Oman, Egypt, Iran

- MECP has decided that discussions must be held in person
- GMP to establish dates for MECP meetings each year well in advance to improve predictability and planning

STOP-Malaria

Global **Malaria** Programme

World Health Organization

Background

- Approaching elimination, resources diverted to more significant public health problems
- Elimination requires good epidemiologic and problem solving skills, focus
- STOP-Polio: WHO and CDC programme to support last mile of polio elimination
  - Mid-career professionals volunteer for 1 year
  - WHO consultants, embedded with MOH at subnational level
  - Standardized training
  - Provided with a daily living allowance
  - Weekly activity reporting
- STOP-Malaria launched in Botswana in August 2019

Components of STOP-Malaria

- Recruitment process to attract trained and experienced public health professionals
- Rigorous training in malaria elimination strategies, mentoring/interpersonal skills
  - 2-week training in Botswana, included WHO and MOH staff for first week
- Prepared field deployment at focus/district level, as appropriate, under WHO umbrella
  - All STOPpers currently deployed in country
- Situation analyses conducted using malaria elimination audit tool
- Weekly monitoring of activities
- Need to improve recruitment of Spanish and Portuguese speakers
- Next cohort to start in May 2020
  - 6-7 STOPpers expected

Thank you!